Detection of Algerian Honey Adulteration by Raman Spectroscopy and Chemometrics Methods

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Summary

Honey is one of the most popular foods in Algeria. This study, Raman spectroscopy combined with chemometrics methods: - principal component analysis (PCA), - hierarchical cluster analysis (HCA) was used to achieve the identification and detection of pure and adulterated honeys. Thus, 16 samples of authentic Algerian honey samples taken in different geographical locations from west and south-west of Algeria and 72 of adulterated samples were each mixed with authentic honey samples in the following ratios: 1: 20 (5%), 1:10 (10%), 1:5 (20%), 1:3 (30%), 1:2.5 (40%) and 1:2 (50%) and fructose, glucose, sucrose and syrup were analyzed. PCA and HCA were successfully used to process spectral data for discrimination of pure honey and adulterated honey, so we showed a successful separation between pure and adulterated honey. We observed clearly three clusters (A: pure honeys, B: adulterated honeys, C: adulterants (Glucose (G), Fructose (F), sucrose (S)). Raman spectroscopy was efficient in discrimination of honey using PCA and HCA. The PC1-PC2 plane, which accounts for 98.34 % of total variance could be sufficient to distinguish authentic honeys. The proposed methods based on Raman spectra have important utility for food safety and quality control of honey products.

Key words

honey, adulteration, Raman Spectroscopy, PCA, HCA

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Introduction

Honey is a syrupy, sweet, naturally occurring material produced by bees. Honey is a sustenance that honeybees make from the nectar of flowers or the secretions of plant parts. Bees collect these ingredients, transform them, mix them with other ingredients, store them, and then allow them to develop in the hive. This product can be thin, solid, or crystalline (Chen et al., 1998, Baglio et al., 2017). The existence of the health benefits of honey in ancient scriptures has been used since antiquity, starting from traditional ancient Egyptians, Chinese, Greeks, and Romans (Chen et al., 1998, Baglio et al., 2017). The chemical composition of honey is very complex (Stanley and Linskens, 2011). Honey is said to contain around 181 substances (El Sohaimy et al., 2015; Bucekova et al., 2019). Honey is an excellent source of energy containing around 80 g 100 g^{-1} of carbohydrates (35 80 g 100 g^{-1} of glucose, 40 80 g 100 g^{-1} of fructose and 5 80 g 100 g^{-1} of sucrose) (Almasaudi et al., 2017) and 20 g 100 g^{-1} of water, quantitatively the second most important component of honey (Terrab et al., 2002). The water content varies between 14 and 25%, depending on the type of honey (Pataca et al., 2007). It is the content that will determine the quality and how the honey is stored. In addition, honey contains organic acids such as acetic acid and gluconic acid, which are responsible for the acidity of honey (Chick et al., 2001). Vitamins and minerals are present in very small quantities, especially iron and copper that are responsible for the redox properties of honey and potassium, being the most abundant (Osman et al., 2011). Honey also contains traces of niacin, calcium, copper, riboflavin, iron, magnesium and zinc (Nanda et al.2003). The main enzymes in honey are invertase, diastase and glucose oxidase (Lazarević et al., 2012). Among the constituents of honey are also amino acids, 5-hydroxymethylfurfural (HMF) (Silva et al., 2009) and phenolic compounds. The flavonoids present in honey are composed of flavanones (Ouchemoukh et al., 2010). Phenolic acids are substituted cinnamic acids and benzoic acids. These compounds are the main contributors to the colour, taste and aroma of honey (Wootton-Beard et al., 2011).

Since ancient times, honey has been of great importance as it was used as food and medicine because it contains a very high percentage of beneficial plant compounds and offers many health benefits. It contains some nutrients (Bogdanov et al., 2012) and is high quality and rich in antioxidants (Dezmirean et al., 2012) and less harmful than sugar for diabetics (Rai et al., 2019). The amount of antioxidants in it helps lower blood pressure (Erejuwa et al., 2011, Chikhi et al., 2019) and is linked to other beneficial effects on heart health (Scepankova et al., 2017). Honey also helps improve cholesterol (Nemoseck et al., 2011), can lower triglycerides (Nurmasitoh et al., 2015), helps heal wounds and burns (Majtan et al., 2014), and helps suppress coughs in children (Goldman et al., 2014). It is delicious but still high in calories and sugar (Curtis et al., 2006) Honey has biological activities such as antibacterial and wound healing activity, anti-inflammatory effects, antioxidant activity (Vallianou et al., 2014).

Since free natural honey is widely used and demanded in several areas, including medicine, the pharmaceutical industry and cosmetics, as well as foodstuffs and is considered as a basic food for some species, given this great importance of free natural honey, as well as the large demand for it by manufacturers, its price is rather high. These advantages help in making adulterated honey by adding human-made components such as glucose, fructose, and sucrose. Honey is considered the third food target in natural products for fraud in the world after milk and olive oil (Zhu et al., 2010). Many researchers in the field of suppressing fraud in natural materials did not overlook these abuses, so they touched on several methods for detecting fraud in honey and several other natural materials, and most of them were successful in differentiating between free natural honey and adulterated honey in it. To determine the purity of honey, several numerous studies have been carried out either by HPLC (Manzanares et al., 2011; Wang et al., 2015) and isotope ratio mass spectrometry IRMS (Cabañero et al., 2006; Simsek et al., 2012) or CPG (Kaškonienė et al. 2011) chromatography methods, by NMR spectroscopy methods (Justino et al., 1998; Bertelli et al., 2010), by infrared spectroscopy (Huang et al., 2020; Sivakesava et al., 2001), or by surface plasmon resonance optical sensor (Zainuddin et al., 2018). All these methods are expensive and are not economical. With a new, innovative and easy-to-use technology, we apply the first method of its kind to detect fraud in natural materials, including honey. Our innovative technology is the use of informatics in chemistry and the combination of both in the so-called chemometric. Among these methods, we use Raman spectroscopy (Oroian et al., 2018; Wu et al., 2022), a vibrational molecular spectroscopy technique based on the detection of scattered photons after the interaction of the sample with a monochromatic light beam. Raman spectroscopy has been used in many fields like detection of materials, detection of bacteria and detection of adulteration in food safety and quality control (Özbalci et al., 2013; Oroian et al., 2018; Wu et al., 2022; Chiali et al., 2022; Belhocine et al.,2023) to measure a group of simple sugars belonging to the carbohydrate family, which are glucose, fructose and sucrose, and we have also used it for pure honeys and honeys adulterated by sugars. Raman spectra were processed using Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA), which is one of the most popular methods for data dimensionality reduction and explorative analysis (Pořízka et al., 2018, Zhao et al., 2013, Tanoh et al., 2020).

Undoubtedly, some studies have been carried out on honey in Algeria (Issad et al., 2021). This is also the case for neighboring countries such as Morocco and Tunisia (Ouradi et al., 2021). However, in view of the importance of this substance, the characteristics remain relatively unknown, and by comparison with studies carried out around the world, the number of articles remains insufficient. In general, Algeria remains understudied in the field of natural sciences, as reported in a recent study (Ghorab et al., 2021) and none of these articles addressed the problem of adulteration of honey with different types of sugars with IRTF investigations. The objective of our work is to define a method of detection based with Raman spectroscopy for the specific Algerian honeys.

Materials and Methods

Materials

High purity (99%) glucose, fructose and sucrose were purchased from sigma-Aldrich, the three sugars were used without any further processing. The syrup sugar, a mixture of glucose, fructose and sucrose syrup, was purchased from Ela factory in the Algerian city of Annaba.

Samples

In this part, the study was carried out on 16 samples of authentic honeys from the south and south-west of Algeria (Fig.1 represents the different geographical regions used to collect authentic honey samples and Table 1 represents geographical location of honey samples from different regions of Algeria), and 72 samples of adulterated honeys.

The honey samples were collected between 2020 and 2021 and stored at 4–6 °C in the absence of light. Samples were mixed well and kept at room temperature to equilibrate before Raman measurements. The regions from which the samples of honey were collected are indicated in Fig. 1 and Table 1.

The adulterated honeys were prepared from pure honeys: we put pure honeys in a small glass jar with well-defined masses and we added the sugars (glucose, fructose, sucrose) that were prepared in advance by dissolving them beforehand in water and also adding another additive, the syrup which was mentioned early. The mixtures were well mixed to obtain a homogeneous mixture at a temperature of 25 °C with mass percentage (w/w) well specified in the following ratios: 1:20 (5%), 1:10 (10%), 1:5 (20%), 1:3 (30%), 1:2.5 (40%), 1:2 (50%).

Raman Spectroscopy

Raman spectroscopic investigation was performed using a HORIBA LABRAM HR Raman spectrometer operated in single spectrograph mode with a holographic dispersive grating of 600 grooves mm^{-1} equipped with a frequency (633, 785 and 325 nm).

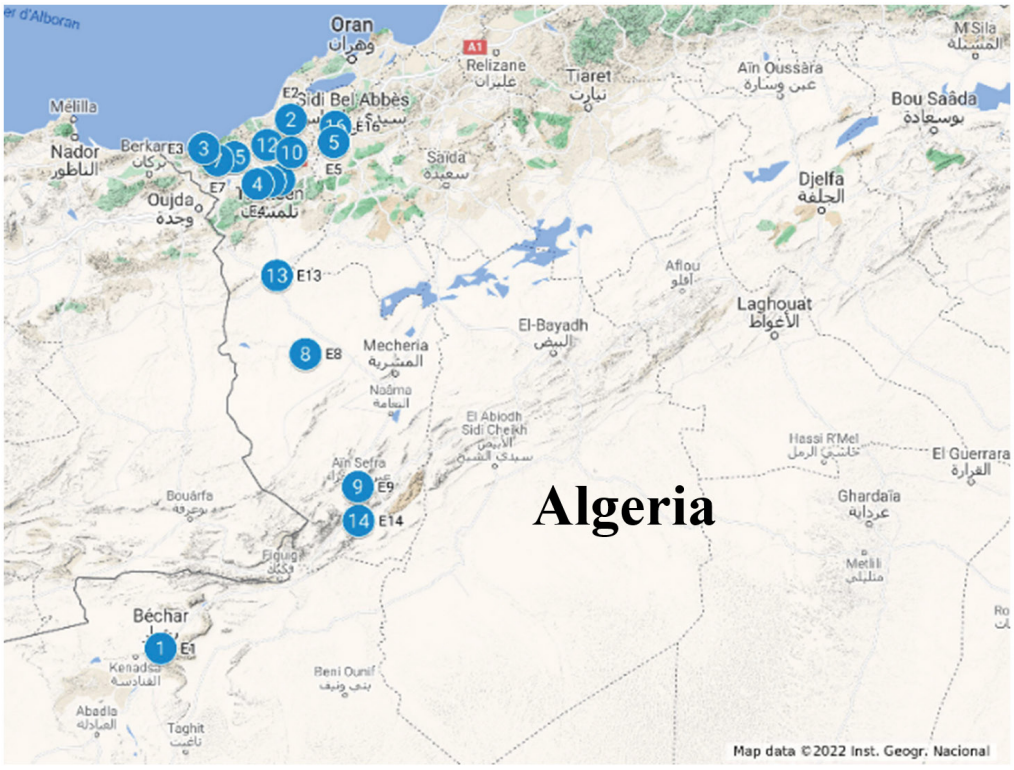
The samples were analyzed in the back-scattering mode on the microscope stage of an Olympus confocal microscope attached to the spectrometer using a long working distance 50× objective. The detector used was a liquid nitrogen cooled charge coupled device Symphony IGA detector. 663 and 785 nm holographic notch filters were used to remove the Rayleigh-scattered light used for sugars analysis and adulterated honeys respectively.

The entrance slit width was 100 µm giving a resolution of 2 cm^{-1} in the range between 0 and 5000 cm^{-1} with 15 accumulations scans and 5s time acquisition. Repeated acquisition using the highest magnification was accumulated to improve the signal to noise ratio in the spectra. Spectra were calibrated using the 520.5 cm^{-1} line of a silicon wafer.

Chemometrics Analysis

We have used a combination of Raman spectroscopy and chemometrics methods including principal component analysis (PCA) and hierarchical cluster analysis (HCA) for detection and discrimination of pure and adulterated honey samples. HCA and PCA were executed in order to evaluate the Raman spectral differences between pure honey samples and adulterated honey samples.

Discrimination and classification of 16 samples of pure honey and 31 samples of adulterated honey were selected among different samples of adulterated honey, where we chose samples of adulterated polyfloral honey and samples of adulterated monofloral honey and we also chose percentages mass (w/w) different in the following proportions: 1:20 (5%), 1:10 (10%), 1:5 (20%), 1:3 (30%), 1:2.5 (40%), 1:2 (50%) with glucose, fructose, sucrose and syrup. All was performed using XLSTAT Version 2014.5.03 software.



1	31°37′00″North, 2°13′00″W
2	35°17′22″North, 1°08′28″W
3	35°05′38″North, 1°51′37″W
4	34°51′00″North, 1°25′00″W
5	35°08′09″North, 0°47′10″W
6	34°51′56″North, 1°19′05″W
7	35°00′47″North, 1°44′51″W
8	33°40′49″North, 1°01′13″W
9	32°45′20″North, 0°35′09″W
10	35°04′00″North, 1°08′00″W
11	34°52′38″North, 1°14′07″W
12	35°07′04″North, 1°20′03″W
13	34°13′22″North, 1°15′21″W
14	32°31′04″North, 0°34′49″W
15	35°02′06″North, 1°36′21″W
16	35°14′35″North, 0°46′23″W

Figure 1. Map of Algeria showing the different geographical regions used to collect authentic honey samples

Table 1. Geographical location of honey samples from different regions of Algeria, vernacular name, scientific name and code of each sample

Code	Vernacular name	Scientific name	Harvest Algerian region	Geographic coordinates
E1	Jarjir	*Eruca vesicaria* (L.) *Cav.*	Bechar	31°37'00"North, 2°13'00"W
E2	Caroub	*Ceratonia siliqua* L.	Ain Temouchent	35°17'22"North, 1°08'28"W
E3	Multifloral 1	Polyfloral	Tlemcen (Ghazaouet)	35°05'38"North, 1°51'37"W
E4	Zaitra	*Thymus vulgaris* L.	Tlemcen (Aindouz)	34°51'00"North, 1°25'00"W
E5	Multifloral 2	Polyfloral	Sidi Bel Abbes (Sidi yacoub)	35°08'09"North, 0°47'10"W
E6	Mountain 1	Polyfloral	Tlemcen (lalla setti)	34°51'56"North, 1°19'05"W
E7	Mountain 2	Polyfloral	Tlemcen (Nedroma)	35°00'47"North, 1°44'51"W
E8	Loubuna	*Euphorbia amygdaloides* L.	El Bayed	33°40'49"North, 1°01'13"W
E9	Sidr 1	*Ziziphus lotus* L. *Desf.*	Naama (Ain Sefra)	32°45'20"North, 0°35'09"W
E10	Sidr 2	*Ziziphus lotus* L. *Desf.*	Tlemcen (Sidi Abdlli)	35°04'00"North, 1°08'00"W
E11	Multifloral 3	Polyfloral	Tlemcen (Ain fezza)	34°52'38"North, 1°14'07"W
E12	Multifloral 4	Polyfloral	Tlemcen (ELFehoul)	35°07'04"North, 1°20'03"W
E13	Harmel	*Peganum harmala* L.	Tlemcen (Aricha)	34°13'22"North, 1°15'21"W
E14	Multifloral 5	Polyfloral	Naama (Moghrar)	32°31'04"North, 0°34'49"W
E15	Multifloral 6	Polyfloral	Fellaoucene tlemcen	35°02'06"North, 1°36'21"W
E16	Multifloral 7	Polyfloral	Sidi Bel Abbes (Tessala)	35°14'35"North, 0°46'23"W

Results and Discussion

Raman Spectra of Sugars, Authentic Honeys and Adulterated Honeys

Fig. 2 shows Raman spectra of sucrose, fructose, glucose and syrup used in adulteration; we also analysed the spectra of pure honeys (Fig. 3) and compared them with falsified honeys (Fig. 4).

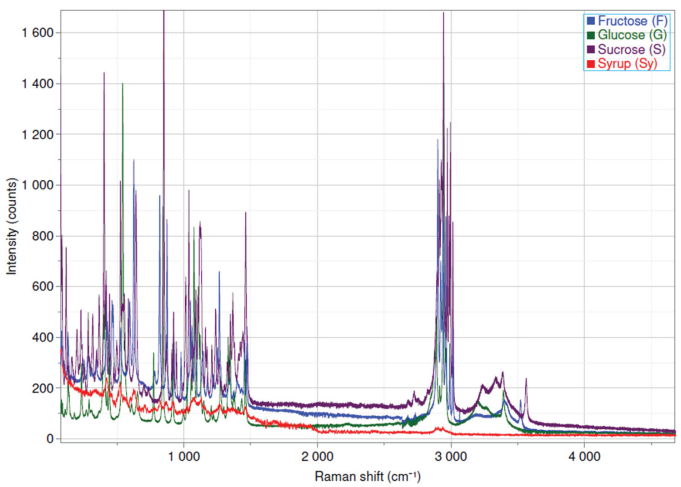


Figure 2. Raman Spectra of: sucrose (S), fructose (F), glucose (G) and syrup (Sy)

Raman spectra of the glucose showed characteristic peaks at 332 and 356 cm^{-1} corresponding to an endocyclic vibration of δ (C-C-C) and δ (C-C-O) respectively, we observed at 441, 540 and 773 cm^{-1} peaks corresponding to the bending vibration δ (C – C – O), the vibration of δ (C – C – C) of carbohydrates and vibration of δ (C – H) and ν (C – C) respectively (Mathlouthi et al., 1980, Šugar et al., 2016). The peak at 842 cm^{-1} is the vibration of υ (C-O-C). The bands at 995 and 1021 cm^{-1} corresponded to the vibration of υ (C – O) in the two enemies (Šugar et al., 2016). The bungs at 1433 and 1459 cm^{-1} corresponded to the vibration off δ (CH_2) and δ (CH_2) asymmetry respectively. At 2946 cm^{-1} was the vibration jump of υ (CH_2) and at 3390 cm^{-1} was the vibration jump of υ (OH) (De Oliveira et al. 2002).

From the fructose we can observe peaks at 312 and 345 cm^{-1} corresponding to an annular δ (C – C – C) vibration of pyranoid and furanoid forms, respectively, then peaks at 421, 623 cm^{-1} corresponding to the vibration of δ (C – C – O) and δ (C – C – C) of carbohydrates repetitively (Šugar et al 2016), at 760 and 816 cm^{-1} we observe the vibration of υ (C – C) in fructopyranose and the υ (C – C) vibration in fructofuranose respectively. The peak at 869 cm^{-1} is the vibration of υ (C-O-C). Large peaks are observed at 1031 and 1045 cm^{-1} corresponding to the vibration υ (C – O) in the pyranoid and furanoid rings. The peaks at 1453 and 1476 cm^{-1} correspond to the vibration of δ (CH_2) and δ (CH_2) asymmetry respectively. From 2899 to 2942 cm^{-1} is the vibration jump of υ (CH) and from 2962 to 3015 cm^{-1} is the vibration jump of υ (CH_2).

The jump of 3529 cm^{-1} corresponds to the vibration of υ (OH) (Šugar et al., 2016, Mathlouthi et al., 1980, Özbalci et al., 2013).

From the sucrose the same annular δ (C – C – C) vibrations of pyranoid and furanoid forms peaks are observed at 315 and 356 cm^{-1} (Mathlouthi et al., 1980). At 345 cm^{-1} we observe the vibration of endocyclic δ (C-C-O). The peaks at 441 and 524 cm^{-1} correspond to the vibration of δ (C – C– O) in the pyranoid ring of fructose and the vibration of the α-glycosidic bond of C1 on the glucosyl subunit respectively (Mathlouthi et al., 1980). At 640 cm^{-1} is the vibration of υ (C – C) of fructopyranose (Mathlouthi et al., 1980) and at 849 cm^{-1} it corresponds to the vibration of δ (C – C – O) and δ (C – C – C) of carbohydrates (Šugar et al., 201,6). The 1035 cm^{-1} band of the sucrose spectrum has been attributed to the vibration of υ (C – O) of the glucose ring (De Oliveira et al. 2002). The jump at 1463 cm^{-1} is the vibration of δ (CH_2) asymmetry. From 2916 to 2942 cm^{-1} is the vibration jump of υ (CH) and from 2950 to 2995 cm^{-1} is the vibration jump of υ (CH_2). The jump of 3563 cm^{-1} corresponding to the vibration of υ (OH) (Šugar et al., 2016, Mathlouthi et al., 1980, Özbalci et al., 2013, De Oliveira et al., 2002).

Finally, from the industrial syrup we observe peak at 421cm^{-1} corresponding to a vibration of δ (C – C – O) of glucose (Mathlouthi et al., 1980). At 522 cm^{-1} it is a vibration of δ (C – C – O) and δ (C – C – C) of carbohydrates (Özbalci et al., 2013). At 633 cm^{-1} there appears a deformation, vibration of the ring, at 782 cm^{-1} there is a vibration of δ (C – H) and ν (C – C) of glucose (Šugar et al., 2016). The jumps at 821 and 863 cm^{-1} correspond to a vibration of δ (C – H), δ (CH_2) and δ (C – O – H). At 980 cm^{-1} we observe the vibration peaks in the two enemies of fructose and glucose. The peak at 1075 cm^{-1} there is a vibration of υ (C – O). At 1121 cm^{-1} it corresponds to a vibration of υ (C – O) and υ (C – O – C) in carbohydrates and υ (C – N) from proteins and amino acids, 1270 cm^{-1} gives a vibration of υ (C – O – H), υ (amide III). At 1351 cm^{-1} comes a vibration of δ (O – H) and δ (C – H). The jump to 1461 cm^{-1} corresponds to a vibration of δ (CH_2) and δ (C– C– O) of flavanols and organic acid. From 2880 to 2908 cm^{-1} is the vibration jump υ (CH) and from 2920 at 2937 cm^{-1} is the vibration jump υ (CH_2). (Šugar et al., 2016, Corvucci et al., 2015).

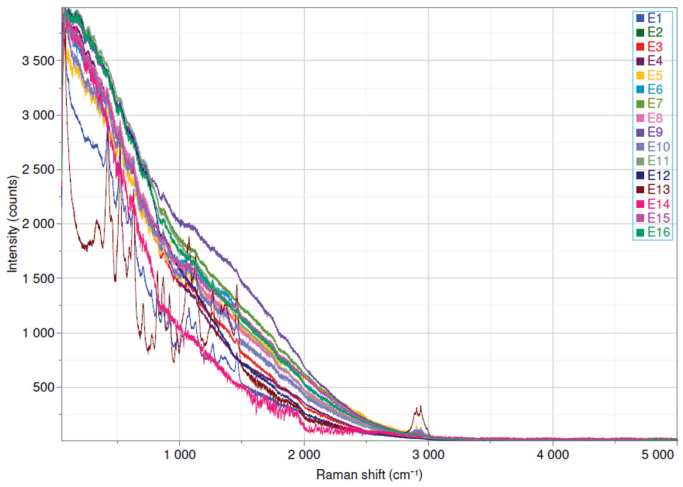


Figure 3. Raman Spectra of E1 to E16 pure honeys

Fig. 3 shows the positions of Raman peaks of pure honeys described in table 1. The main characteristic bands in the spectrum of pure honeys. They are found to be correlated with all three sugars (glucose, fructose, sucrose); it is observed that the large bands of glucose and fructose dominate in the spectra of pure honeys and the band of sucrose is weak. The area between 200 and 1500 cm^{-1} is the most important fingerprint region and area in the spectra (Fig. 2), containing different vibrational modes of bonds like carbohydrates, proteins and organic acids. Sugars are major constituents in honeys; the following table 2 represents the characteristic vibrations peaks and the assignments of all authentic honey samples (Mathlouthi et al., 1980, Šugar et al., 2016, Corvucci et al., 2015).

Raman spectroscopy can be successfully used to detect adulteration of honey (Fig. 4). Raman technique has been applied at honeys to identify and quantify sugars (glucose, fructose, syrup and sucrose contents) and further to characterize them as adulterants. The characteristic spectral bands that correlated to sugars of honey were ≈ 314, 341, 415, 530, 617, 744, 776, 790, 838, 856, 911, 933, 1028, and 1106 cm^{-1} (Sotiropoulou et al., 2021, Zhu et al., 2010). Moreover, Raman technique was used by Salvador et al (Salvador et al., 2019, Gok et al., 2015). to detect the sugar content and the type of adulteration in commercial honeys. The main observed bands of honeys from caroub (E2) were 312, 332, 345, 453, 704, 800, 1081, 1062, 1362 cm^{-1}. These bands were assigned to the presence of sugar (glucose, fructose, and sucrose) in honey samples.

The bands of authentic honey at 704 and 800 cm^{-1}, with sucrose adulteration, were overlapped with strong absorptions at 822 and 834 cm^{-1}. Principal component analysis was applied and confirmed the applicability of Raman technique for the detection of adulteration in honey with glucose, fructose, and sucrose.

In another study, Raman spectroscopy was also used to detect adulteration of honey with high syrup. The characteristic bands corresponding to authentic and adulterated honeys were observed: 351, 421, 633, 592, 782, 802, 863, 980, 865, 1121, 1270, 1351, and 1461 cm^{-1}, at the band of 2791 cm^{-1}, while at 1130 cm^{-1}, the absorption was reduced due to the decrease in protein and amino acid content in the adulterated honeys (Sotiropoulou et al., 2021). The Raman spectra of all adulteration honey for all authentic honey samples presented in Table 1 are shown in ESI (Fig. 4).

Chemometric Analysis

In this study, Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) were successfully used to process spectral data for the discrimination of pure and adulterated honeys. We took the values of the most intense peaks in the Raman spectra (spectra of pure honeys, spectra of falsified honeys and spectra of sugars) where we took the value of the intensity of the same peak for all the Raman spectra and then we introduced these data into XLSTAT software, to perform the Principal Component Analysis (PCA) and the Hierarchical Cluster Analysis (HCA). Hierarchical Cluster Analysis (HCA) allows building a hierarchy of clusters.

In HCA, clusters and sub-clusters are clearly visualized in dendrogram chart. HCA for discrimination of pure honey and adulterated honey samples is presented in (Fig. 5). The visualization of dendrogram revealed that pure honeys and adulterated honeys test samples were clearly discriminated. As can be seen from the HCA dendrogram, that samples were mainly classified as two main clusters (the left side presents adulterated honeys samples whereas the right side presents pure honeys samples). The two clusters were clearly distinguished and also were observed with high heterogeneity value. On the other hand, Principal Component Analysis (PCA) was executed for pure honeys and adulterated honeys samples. The result of PCA is presented in (Fig. 6). PCA results showed a successful separation between pure and adulterated honeys. We observed clearly three clusters (**A**: pure honeys, **B**: adulterated honeys (Table 3), **C**: adulterants (glucose, fructose and sucrose)).

Chemometrics tools frequently used to classify data are the Principal Component Analysis and Hierarchical Cluster Analysis. With these methods you do not require any previous sample preprocessing and can provide patterns, subspaces, groupings, classes (Zhao et al., 2013, Tanoh et al., 2020, Chaker et al., 2021). These techniques were applied to the Raman shift of the different regional zones considering normalized Raman spectra to the maximum intensity and considering the range as well between 0 to 5000 cm^{-1}.

To the best of our knowledge, this present research is the first attempt for detection and discrimination of pure and adulterated honey samples from North Africa using Raman spectroscopy coupled with chemometrics analyses (PCA and HCA). These tools were successfully applied to discriminate authentic honeys from fraudulent samples on the basis of Raman spectra. Several reports dealt with spectroscopic methods (ATR-FTIR, Raman, UV-Vis, Carbon isotope ratio ($^{13}C/^{12}C$)) for detection of adulteration in honey based on chemometrics methods (Gok et al., 2015, Li et al., 2012, Yulia et al., 2017, She et al., 2019, Chikhi et al., 2019). Other research used chromatography methods (GC, HPLC) for analysis of adulteration in honey (Manzanares et al., 2011, Kaškonienė et al., 2011).

Table 2. Raman vibration bands assignments of authentic honey samples

Peak position (cm^{-1})	Intensity	Vibrational assignments
312	very weak	δ (C – C – C) annular of pyranoid forms of fructose 31
332	very weak	δ (CCC) endocyclic of glucose 31
345	very weak	δ (C – C – C) annular of furanoid forms of fructose 31
356	very weak	δ (CCO) endocyclic of glucose 31
419 to 453	shoulder	δ (C – C – C) ring of carbohydrates (glucose and fructose) 35,36
515	stronger	δ (C – C – O) and δ (C – C – C) of carbohydrates 32
704	weak	υ (C – C) in fructopyranose 33
779	weak	δ (C – H) and ν (C – C) of glucose 32
820	weak	υ (C – C) in fructofuranose [33]
869	medium	δ (C – H) and δ (CH_2), δ (C – O – H) 32
918	medium	δ (C – H) and δ (C – OH) 32
1078	strong	υ (C – O) in the two enemies 31
1081	strong	δ (C – H) and δ (C – O – H) from carbohydrates and δ (C – N) from proteins and amino acids 32
1128	strong	υ (C – O) and υ (C – O – C) from carbohydrates and υ (C – N) from proteins and amino acid 32
1266	strong	υ (C– O – H), υ (amide III) 37
1362	weak	δ (O – H) and δ (C – H) 32,37
1462	medium	δ (CH_2) and δ (CCO– of flavanols and organic acid) 32
2885 to 2905	very weak	υ (CH)
2930 to 3000	very weak	υ (CH_2)

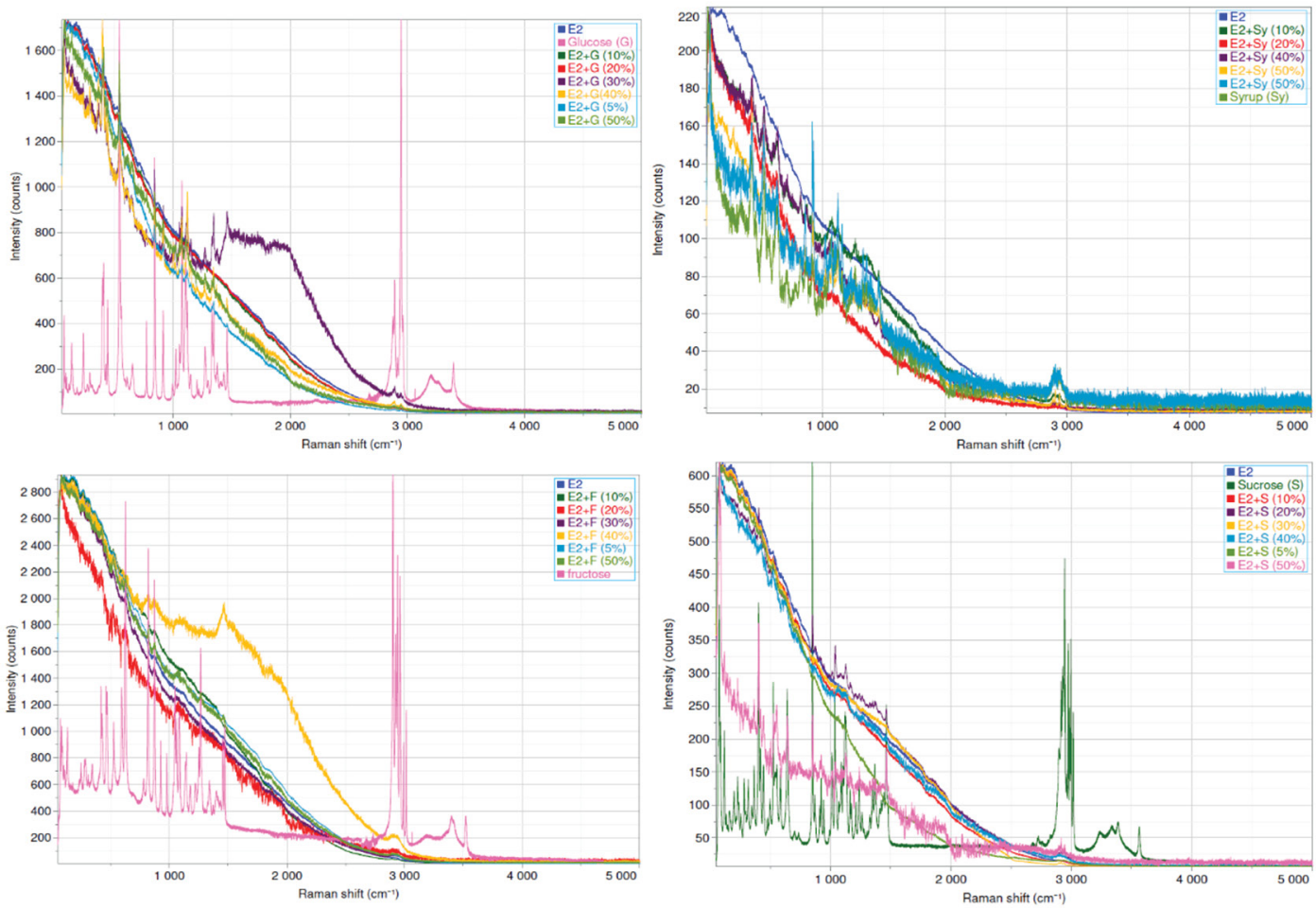


Figure 4. Raman spectra of adulteration of authentic caroub honey (E2) by sucrose (S), glucose (G), fructose (F) and syrup (Sy) with different ratio (5%, 10%, 20%, 30%, 40% and 50%)

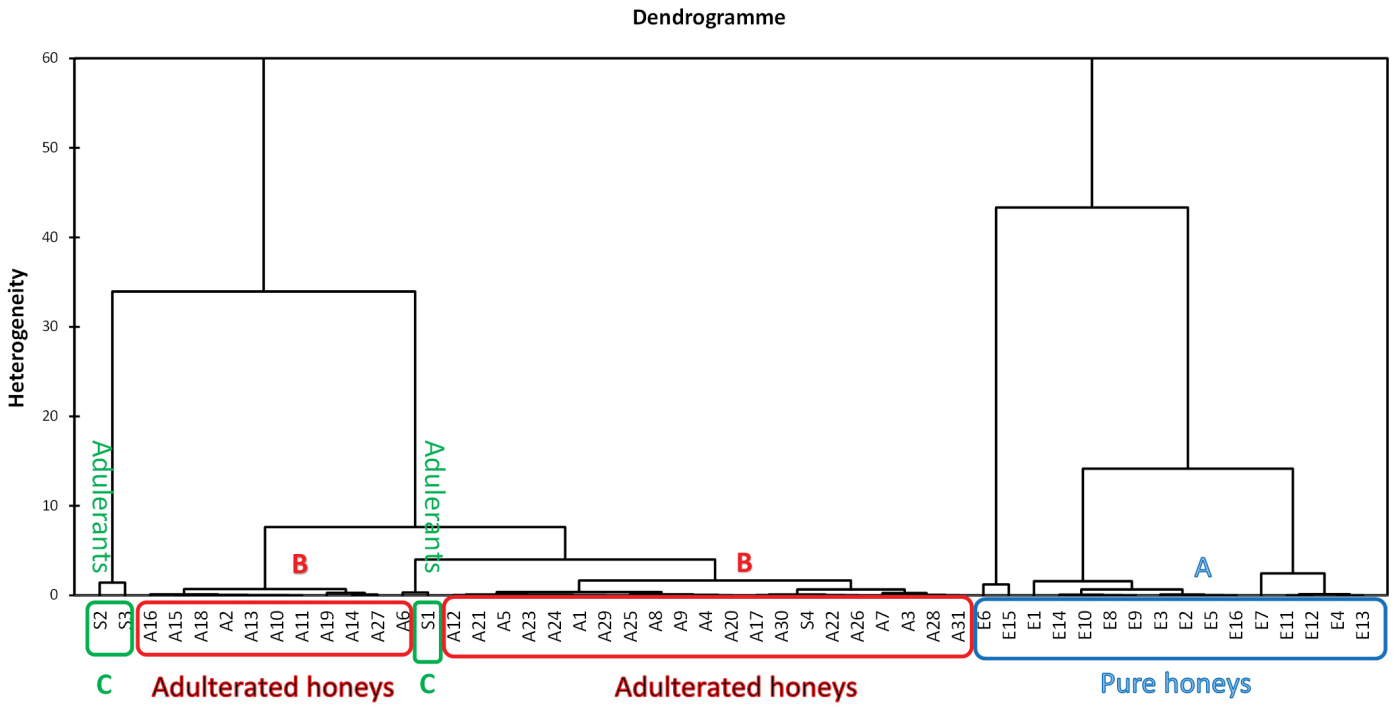


Figure 5. HCA for discrimination of pure honey, adulterated honey samples and sugars

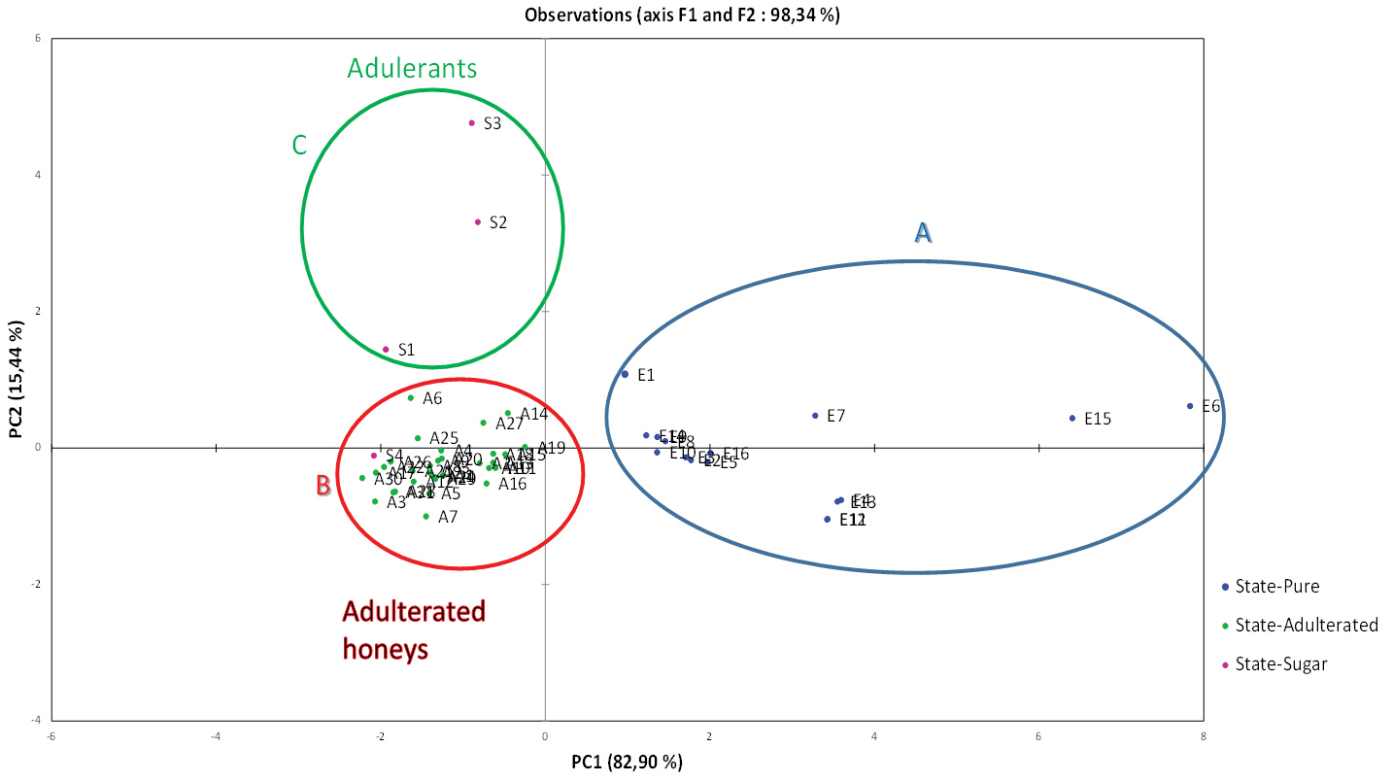


Figure 6. PCA for discrimination of pure honey, adulterated honey samples and adulterants (sugars)

Table 3. Adulterated honey and its code

Code	Adulterated honey	Code	Adulterated honey
A1	E2 + G (5%)	A17	E7 + S (40%)
A2	E2 + G (10%)	A18	E7 + S (50%)
A3	E2 + G (20%)	A19	E10 + Sy (5%)
A4	E2 + G (30%)	A20	E10 + Sy (10%)
A5	E2 + G (40%)	A21	E10 + Sy (20%)
A6	E2 + G (50%)	A22	E10 + Sy (30%)
A7	E3 + F (5%)	A23	E10 + Sy (40%)
A8	E3 + F (10%)	A24	E10 + Sy (50%)
A9	E3 + F (20%)	A25	E12 + S (20%)
A10	E3 + F (30%)	A26	E12 + G (30%)
A11	E3 + F (40%)	A27	E12 + F (40%)
A12	E3 + F (50%)	A28	E12 + Sy (50%)
A13	E7 + S (5%)	A29	E14 + S (50%)
A14	E7 + S (10%)	A30	E14 + F (50%)
A15	E7 + S (20%)	A31	E14 + G (50%)
A16	E7 + S (30%)		

The result of PCA (Fig. 6) showed a clear cluster separation of pure honeys, adulterated honeys and adulterants (glucose, fructose and sucrose). The first principal component (PC1) explained 82.9 % of the total variance; the second 15.44 %; these two components PC1-PC2 plane could be sufficient for distinguishing authentic honey. We observed that all of the pure honey samples were located at the positive side of PC1 score scale and the adulterated honeys samples were located at the negative side of the same scale. The PC1-PC2 plane, which accounts for 98.34% of total variance could be sufficient for distinguishing authentic honey. Furthermore, the results of this study show that Raman spectroscopy techniques combined with hierarchical cluster analysis have shown high capability for detection of adulterated honeys. In accordance with the PCA results in the HCA dendrogram (Fig. 5), we observed tow clusters. All of the pure honey samples were heaped together in one cluster at the right side of the HCA, while adulterated honey samples were clustered at the left side. Sides were noted as B and C on the dendrogram, respectively. In conclusion, the results from two different chemometrics classification methods (HCA and PCA) were quite coherent with each other.

Conclusion

The combination of Raman spectroscopy with chemometric methods (PCA and HCA) offers a valuable technique for the discrimination of pure honey. This method has a high potential and capacity to differentiate pure honey from adulterated honey, especially in Algeria, which is new in this field. This method is simpler, easier and faster. Thanks to these new analytical techniques, it has been possible to quickly discriminate pure honey, especially for routine honey analysis.

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Supporting information

Detection of Algerian honey adulteration by Raman spectroscopy and chemometrics methods

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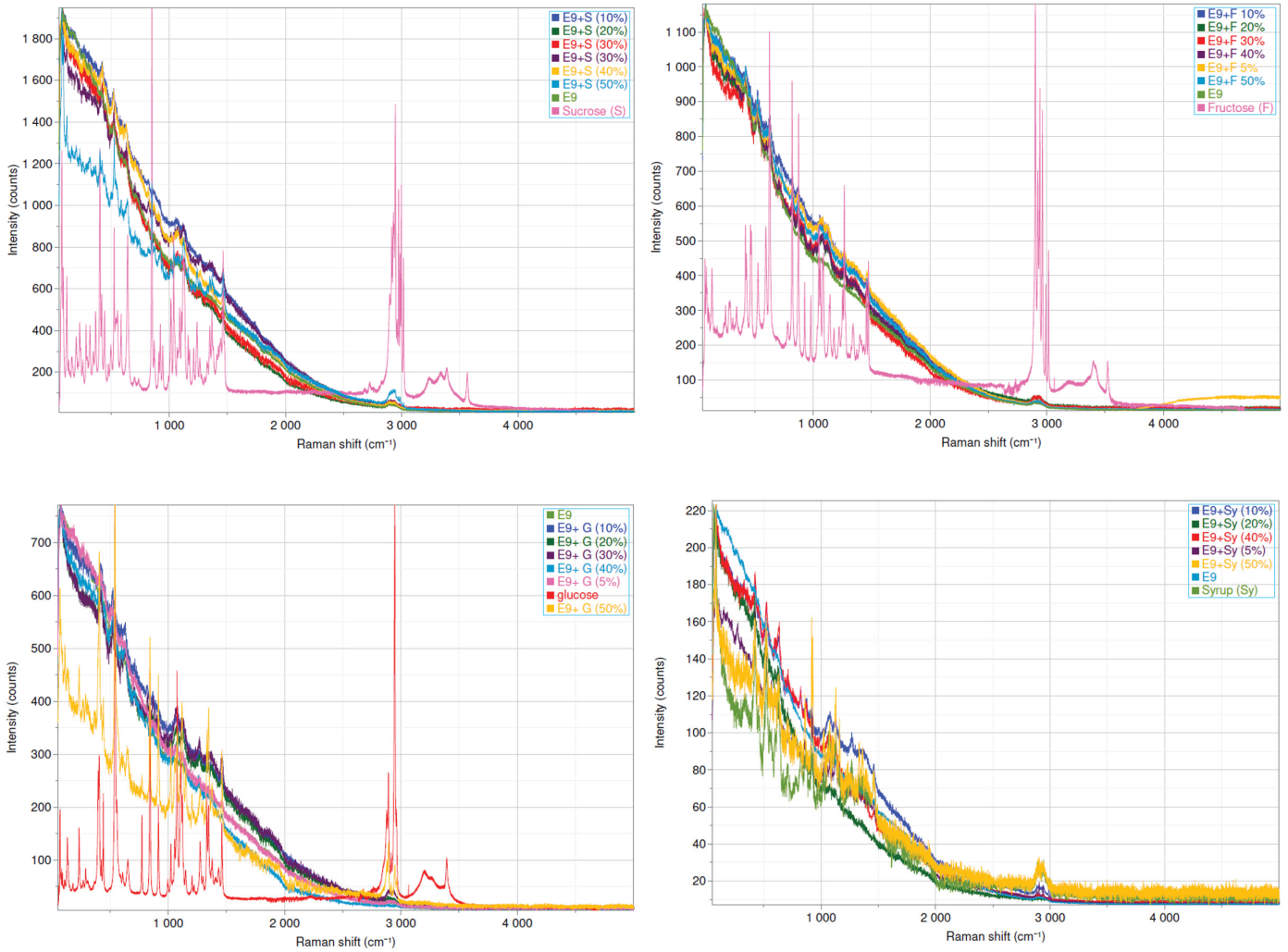

Figure SI 1. Raman spectra of Adulteration of authentic Sidr honey E9 by Sucrose (S), Glucose (G) and Fructose (F) with different ratio (5%, 10%, 20%, 30%, 40% and 50%)

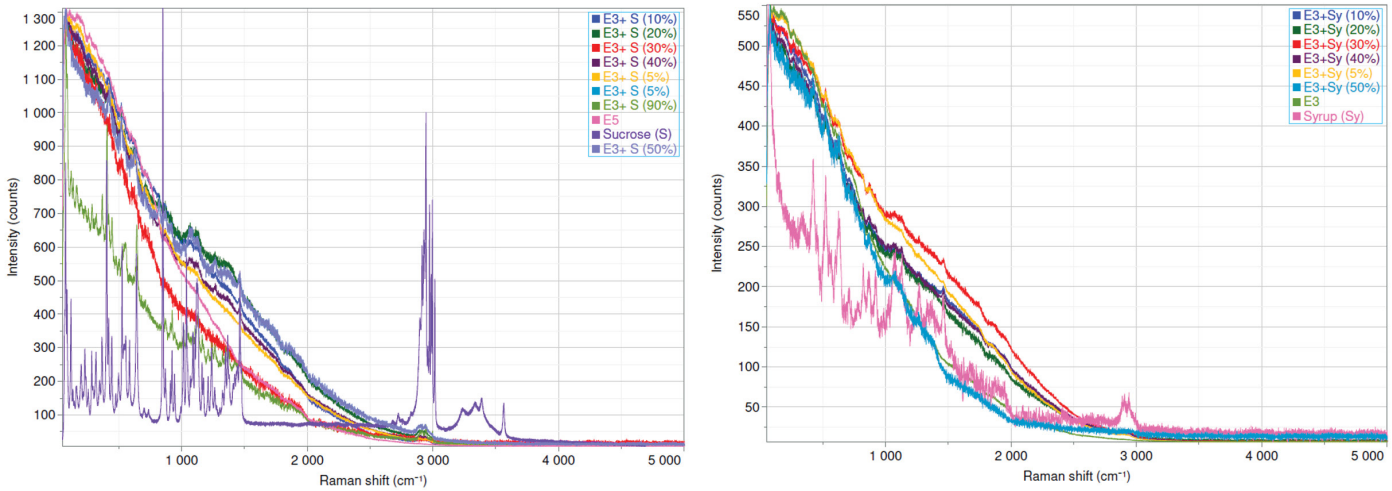

Figure SI 2. Raman spectra of Adulteration of authentic Multifloral 1 honey E3 by Sucrose (S) and Syrup (Sy) with different ratio (5%, 10%, 20%, 30%, 40% and 50%)