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Monofloral honey authentication by voltammetric electronic tongue: A comparison with ^{1}H-NMR spectroscopy

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Abstract

Proton-nuclear-magnetic-resonance-spectroscopy (^{1}H-NMR) is the widely accepted reference method for monitoring honey adulteration; however, the need to find cheaper, faster, and more environmentally friendly methodologies makes the voltammetric-electronic-tongue (VET) a good alternative. The present study aims to demonstrate the ability of VET (in comparison with ^{1}H-NMR) to predict the adulteration of honey with syrups. Samples of monofloral honeys (citrus, sunflower and heather, assessed by pollen analysis) simulating different levels of adulteration by adding syrups (barley, rice and corn) from 2.5 to 40% (w/w) were analyzed using both techniques. According to the indicators (slope, intercept, regression coefficient-R^2, root mean square error of prediction-RMSEP) of the partial-least-squares (PLS) regression models, in general terms, the performance of these models obtained by both techniques was good, with an average error lower than 5 % in both cases. These results support the use of VET as a screening technique to easily detect honey adulteration with syrups.

1. Introduction

Honey is one of the foods with a considerable risk of fraud. This is mainly due to its high composition of sugars, where the adding of syrups can be done quite easily. This fraud tends to affect primarily monofloral honeys since their higher price in the market produces a greater profit margin. Consumers are willing to pay more for a monofloral honey with attributed therapeutic properties such as the antioxidant activity in heather honey (Silva, Chisté, & Fernandes, 2021) or the specific sensory nuances like citrus honey (Juan-Borrás, Periche, Domenech, & Escriche, 2015; Escriche, Juan-Borrás, Visquert, Asensio-Grau, Valiente, 2021; Seraglio, et al., 2021).

In general, a honey is considered as monofloral if the pollen grain percentage is higher than 45% (e.g. Echium sp., Erica sp., Eucalyptus sp., Prunus sp., or Rubus sp.); although, some exceptions exist regarding under/over-represented pollen grains (e.g. Citrus sp., Lavandula sp., Trifolium sp., and Castanea sativa sp. honeys which need at least 10%, 15%, 70%, and 90%, respectively) (Silva, Gonçalves, Nunes & Alves, 2020). On the other hand, a honey should be considered multifloral if it does not meet the pollen, physicochemical or sensory requirements to be considered monofloral (Council Directive 2001/110 Relating to Honey, 2002).

Adulteration is an unfair competition and implies a certain destabilization in the honey markets, hence affecting all beekeepers. Moreover, the consumers may feel deceived when buying a honey that does not meet their expectations in terms of organoleptic flavors and therapeutic characteristics attributed to the pure monofloral honeys; and not to mention the possible toxicological connotations derived from the adulteration practices.

The technique routinely conducted to classify monofloral honeys is the melissopalynological analysis performed by optical microscopy which focuses on the identification of the pollen grains morphology of the different botanical species visited by the bees. However, with this methodology it is not possible to determine whether a syrup has been added to honey, since the sediment to be observed under the microscope would not change, even if the adulteration was done in a significant amount (Louveaux, Maurizio, & Vorwohl, 1970; Juan-Borrás et al., 2015).

Different analytical methods have been tested with the aim of identifying adulteration in the honey, highlighting among others: Fourier reflectance, infrared spectroscopy, high performance liquid chromatography (HPLC) (Wang, et al., 2015; Wu, et al., 2017), carbon ratio isotopic mass spectrometry (SCIRA) (Tosun, 2013), differential scanning calorimetry (DSC) (Sobrino-Gregorio, Vargas, Chiralt & Escriche, 2017). However, the most valued technique, for both the scientific community and the commercial transactions, is the proton nuclear magnetic resonance spectroscopy (^{1}H-NMR). This technique offers a comprehensive range of information on honey, permitting both the

quantification of specific substances related to its quality, such is the hydroximethyl furfural, or the presence of adulterants, like syrups or sugars (Bertelli et al, 2010; Boffo, Tavares, Tobias, Ferreira, & Ferreira, 2012).

Despite the many advantages of ^{1}H-NMR spectroscopy (easy sample preparation; fast acquisition in less than 5 minutes; identification of unknown compounds at molecular level; good repeatability and reproducibility, etc.) (Günther, 2013), its disadvantages (extremely high cost and highly skilled personnel required) hamper the use of ^{1}H-NMR spectroscopy as a routine technique. Therefore, the beekeeping sector is forced to subcontract this expensive service to specialized laboratories, which indirectly results in increased costs of honey for the consumer. Consequently, this sector demands suitable analytical techniques to meet their requirements.

Sensitive to all these issues, the European Parliament (Directive 2014/63/EU, amending Council Directive 2001/110/EC relating to honey, 2002) highlights that to guarantee fair commercial practices and protect the interests of consumers, it is necessary to establish appropriate analysis methods to verify whether honey is compliant with international standards (approved by the Codex Alimentarius). It also indicates that these methods, both those currently recognized and validated and those that may arise as a result of technical progress must be considered for this purpose (Council Directive 2001/110 Relating to Honey, 2002). These new methods should help to identify the authenticity of honey without losing the perspective of the industry requirements (nonspecialized workers, cheap hardware coupled with simple, quick and easy techniques, among others).

The electrochemical techniques, with a rapid response and low-cost, are analytical tools in the forefront of the methods that fulfil these requirements and having the additional advantage of being environmentally friendly. Among them, the electronic tongues based on cross-sensitivity sensors of low selectivity combined with pattern recognition or multivariate analysis tools, are widely used (Lolli, Bertelli, Plessi, Sabatini, & Restani, 2008; Riul, Dantas, Miyazaki, & Oliveira, 2010). This methodology has been widely applied in the classification (Wei, Yang, Wang, Zhang, & Ren, 2018)

or analysis adulteration (Carpintero Barroso de Morais, Ribeiro Rodrigues, Teixeira de Carvalho Polari Souto & Lemos, 2019) of foodstuff. In the specific case of honey, it has been proven to be useful in differentiating honeys in different situations: botanical origin (Escriche, Kadar, Domenech, & Gil-Sánchez, 2012; Pauliuc, Dranca, & Oroian, 2020), geographical origin (Sobrino-Gregorio, Tanleque-Alberto, Bataller, Soto, & Escriche, 2020) and antioxidant capacity (Juan-Borrás, Soto, Gil-Sánchez, Pascual-Maté, & Escriche, 2017). A good correlation between the electronic tongue and different quality physicochemical parameters was found (Escriche, et al., 2012; Juan-Borrás, et al., 2017; Sobrino-Gregorio et al., 2020). In one of our previous works the potential of a voltammetry electronic tongue system to differentiate among different types of pure honey, as well as the addition of syrups at several levels was explored (Sobrino-Gregorio, Bataller, Soto, & Escriche, 2018). Nevertheless, in order to prove the reliability of this electronic tongue in differentiating honey adulterations it necessary to perform an additional validation by comparing it with the reference technique of choice, which is currently ^{1}H-NMR spectroscopy.

Therefore, the aim of this study is to compare the information generated by a voltammetric electronic tongue (VET) with ^{1}H-NMR spectroscopy by using a set of three types of monofloral honeys and simulating different levels of adulterations with several types of syrups. In other words, this research is intended to evaluate the extent to which the results acquired by VET are comparable with those obtained with the established and accepted ^{1}H-NMR spectroscopy.

2. Materials and methods

2.1. Honeys: pollen analysis and sample preparation

Different raw monofloral honeys were used in this study: citrus (*Citru*s spp.), sunflower (*Helianthus annuus*) and heather (*Erica spp.*). Three batches of each type of honey were directly bought from beekeepers in different regions in Spain in 2019. They were classified on arrival at the laboratory by melissopalynological analysis (under the microscope Zeiss Axiolab, Göttingen, Germany at 400x magnification) to corroborate their botanical origin (Escuredo, Silva, Valentão, Seijo & Andrade,

2012; Tanleque-Alberto, Juan-Borrás, & Escriche, 2019). All the samples showed no signs of alteration or granulation and the organoleptic characteristics corresponding to these specific three types of monofloral honey. Furthermore, their physicochemical characterization together with the pollen information is shown in Table S1. After, samples were preserved at 12 °C until they were analysed by VET and ^{1}H-NMR spectroscopy techniques.

A honey was considered to be from citrus if the percentage of pollen from *Citrus* spp. was not lower than 10%; from sunflower, if the pollen from *Helianthus annuus* was not lower than 30% and from heather if the pollen from *Erica* spp. was not lower than 37-45% (Persano-Oddo & Piro, 2004; Von Der Ohe, Persano-Oddo, Piana, Morlot, & Martin, 2004). A new image labelling and annotation software, developed by the Institute of Industrial Computing and Control Systems (AI2) at the *Universitat Politècnica de València*, was used to sample, count and classify the honey pollens. Figure S1 (Supplementary material) shows examples of different photomicrographs corresponding to them.

With the aim of simulating the adulteration of these honeys, three different syrups were used: barley (Finestra Cielo, Italy), rice (Mitoku Macrobiotic, Japan) and corn (Roquette Laisa SA, Spain) which were mixed at different proportions with the three monofloral honeys (ratios honey/syrup in weight: 60/40; 80/20; 90/10: 95/05; 97.5/2.5). In summary, a total of 51 samples (3 monofloral honeys x 3 different syrups x 5 levels of adulteration + 3 pure honeys + 3 pure syrups) were evaluated by VET and ^{1}H-NMR spectroscopy techniques.

2.2. ^{1}H-NMR spectroscopy study

2.2.1. Sample preparation and spectra acquisition

Mixture solutions of each type of honey and adulterant previously mentioned were prepared in dimethylsulfoxide-d_6 (Acros-Organics, New Jersey, USA) (DMSO-d6) with a final volume of 1 mL (Bertelli, et al., 2010). After the preparation, the samples were immediately analysed by 1D ^{1}H-NMR spectroscopy. 700 µL of each sample (mixes and pure honey and syrups) were transferred to a 5 mm NMR tube, (Wilmad Economy grade for 400 MHz). ^{1}H-NMR spectra were performed on a 400 MHz

NMR spectrometer (Bruker Ascend 400) equipped with an ATM 5mm probe (BBO 400 MHz 5mm Z-Grad). Experiments were carried out at 300 K. The experiments consisted in a 1D ^{1}H sequence using a 30-degree flip angle (zg30 Bruker library). Eight scans were performed, with an acquisition time of 4.09 s, spectral width of 5 ppm (2003 Hz), a fid resolution of 0.25 Hz and a size of fid of 16 k. Each spectrum was recorded in 1 min and 39 s.

2.2.2 Spectra processing and assignment

After the spectra acquisition, the free induction decay (FID)'s were Fourier transformed, and their phase, baseline and chemical shift corrected with MestReNova version 6.0.2 (Mestrelab Research SL, Santiago de Compostela, Spain). DMSO signal was used for chemical shift reference to align all the spectra, assuming a chemical shift of this signal of 2.5 ppm relative to tetramethylsilane (TMS). The phase and the baseline correction were performed manually. Resonances were identified according to the bibliography (Bertelli, et al., 2010, Spiteri, et al., 2015). Once processed, the spectra were included in one file to be exported for statistical analysis. Peak intensities were used for semiquantitative approximation.

2.3 Voltammetric electronic tongue data acquisition, data processing

The electronic tongue body (VET) consists of an array of four working electrodes or noble metals (Au, Pt, Ir, Rh) with a purity of 99.9% and 1 mm diameter from Aldrich, housed inside a stainless steel cylinder. The different wire electrodes were fixed inside this cylinder using an epoxy RS 199-1468 polymer. In all cases a calomel electrode was used as reference electrode and the stainless-steel piece was used as a counter electrode. This device included an innovative electrochemical polishing of the working electrodes previously described by Sobrino-Gregorio et al., 2020. Specific in-house software designed by Campos et al., 2013 permitted control of the pumping system and the measurement of the equipment.

Previous dilution of the sample in water (up to 50 mL) was only necessary to start the voltammetric analysis. For each iteration 8 g of honey excluding water (in dry matter) was weighed. After

measurement in each sample, the active surface of the electronic tongue device was self-polished to regenerate the working electrodes active surface. All samples were measured three times and in random order to minimize possible error due to memory of the electrodes.

The voltammetry measurements were carried out on a potentiostatic electronic tongue designed at the Universitat Politècnica de València, Interuniversity Research Institute for Molecular Recognition and Technological Development (IDM) (Alcañiz, et al., 2012). In this work, 40 pulses of 50 ms were applied. The typical distribution of voltages in increasing or decreasing steps of 200 mV between +1 V and −1 V (to avoid water electrolysis) was reported by Sobrino-Gregorio et al., 2018. The potential was set to zero after each potential increase.

2.4. Statistical analysis

Least significant difference (LSD) at significance level α = 5% was used to analyse the differences between samples data. A full residual analysis was previously carried out to check the suitability for all the datasets. In this respect, independence (each sample was randomly selected and independent), homoscedasticity (by means of Levene's test), and normality (by means of a normal probability plot) were all tested. Multivariate statistical analysis, Principal component analysis (PCA) and Partial Least Square (PLS) was used to analyse the VET and ^{1}H-NMR spectroscopy data obtained, and it was performed using the software Toolbox Solo 8.9 (2021, Eingenvector Research, Inc. Manson, WA USA 98831; software available at http://www.eigenvector.com) for chemometric analysis. PCA was applied to evaluate the possible classification (non-supervised) of the pure and adulterated honey samples. Partial Least Square (PLS) was used to generate models to predict quantitative information on the content of honey adulterant in the analysed samples. For the 1H-NMR data analysis, only the spectral regions containing meaningful resonances were included (spectral regions ranging from 2.75 to 3.27 ppm and from 3.45 to 6.7 ppm). The VET and ^{1}H-NMR data were separated in sets according to the adulterant. Each set contained all the data from samples for the three honeys and one adulterant in the different concentrations. So, three initial and independent data sets were built for VET and ^{1}H-NMR

spectroscopy data. Afterwards, in each of these three independent sets, the data from samples regarding two honeys were selected to build a model for the adulterant quantification (training set) and the data for the third honey with adulterant were used to evaluate the performance of this model (validation). This procedure was repeated changing each honey in the validation set for one honey in the training set, and also for each adulterant. Models' performance was evaluated by comparing the correlation coefficient (R^2), a, b (from the simplest linear model: $y = ax + b$) and the root mean square error of prediction (RMSEP) as the most common metric obtained to measure accuracy of this methodology in the representation of predicted vs real level of adulterant in the validation set**.**

3. Results and discussion

3.1. ^{1}H-NMR spectroscopy analysis in pure and adulterated honeys

The ^{1}H-NMR spectra of pure honeys and syrups analysed in this study are presented in Figure 1. The most typical resonances showed in ^{1}H-NMR spectra belong to sugars such as maltose, glucose and fructose. They were identified according to their ^{1}H chemical shift and J-coupling, and compared with previously published literature (Lolli et al., 2008). From downfield shifts, signals from α- and β-maltose (δ_H 6.65 and 6.32 ppm) could be observed only in adulterant syrups or in high percentage-adulterated honey. Moreover, the anomeric hydroxyls of β-glucopyranose at δ_H 6.58, and α-glucopyranose at δ_H 6.18 ppm are also shown in this region. Hydroxyls and anomeric hydroxyls of fructose can be found at δ_H 5.68 ppm (α-fructofuranose), δ_H 5.35 ppm (β-fructofuranose) and δ_H 5.20 ppm (β-fructopyranose). Anomeric protons of glucose can be observed at δH 4.81 ppm for β-glucopyranose and δH 4.74 ppm for α-glucopyranose. The intermediate region between δ_H 3.00 and 4.20 ppm contains the signal of aliphatic protons except for anomeric protons. As should be noted, there are common regions and signals in the ^{1}H-NMR spectrum among monofloral honeys, regardless of botanical origin.

Compared to the typical ^{1}H-NMR spectrum of a pure honey, an adulterated honey presents some hallmarks. Remarkably, the ^{1}H-NMR spectrum of the barley and rice pure syrups exhibited some

226 unresolved resonances, due possibly to their higher viscosity or the presence of undissolved particles.
227 The signals highlighted with dotted boxes in Figure 1 appear with high intensity in the adulterated
228 honey spectrum and in very low intensity, almost at noise level, in the pure honeys spectra. In
229 particular, the signals from anomeric hydroxyls of α- and β-maltose centered at δ_H 6.65 and 6.32 ppm
230 are evident, similar to signals ascribed to other hydroxyls of maltose, sucrose, or other oligosaccharides
231 at δ_H 5.40, 5.00 ppm. Accordingly, these signals grow in intensity from pure honey to pure adulterant
232 throughout the series of different adulterant concentration. An example of these increasing intensities
233 signals by means of a superposition of the spectra involved is shown in Figure 2 that shows ^{1}H-NMR
234 spectra of pure citrus honey, citrus honey adulterated with different concentration of rice syrup and
235 pure rice syrup. The signal at δ = 6.32 ppm has been assigned to the anomeric hydroxyl of β-maltose
236 and has increasing intensity according to the growing content of adulterant syrup.

237 **3.2. Electronic tongue in pure and adulterated honeys**

238 In order to evaluate from a descriptive point of view the global effect of the adulteration level of the
239 three monofloral honeys on the VET data, a Principal Component Analysis (PCA) was performed with
240 the average values of each one of the 51 sample conditions (explained in the section 2.1). This
241 unsupervised procedure confirmed that there was a clear spontaneous classification from the data
242 obtained according to the type of monofloral honey and the level of adulteration. To observe more
243 easily the influence of the addition of the different proportions of syrup to honey, Figure 3 shows, as
244 an example the PCA obtained only for the simulation adulteration with barley syrup. PC1 and PC2
245 explain 51% and 22% of total variance. The first component is more related to the level of adulteration
246 and the second with the type of monofloral honey. In general, the increase in adulteration level
247 promoted a movement towards the left quadrant with an effect more marked for the highest
248 adulteration level (40% of syrup). For each adulteration level, each monofloral honey exhibited
249 different behaviour in terms of the voltammetric measurement.

This figure illustrates that pure and adulterated honeys are detected by VET, since this technique can identify the presence of both electroactive, oxidizable or reducible compounds (associated with the so-called Faradaic current) and dissolved ionic species (which generate the so-called Non-Faradaic current). In the first case, the high content of sugars (monosaccharides and oligosaccharides such as glucose, fructose and maltose) could assume a marked antioxidant character in all these matrices. However, due to the acidic character of honey (pH less than 4) and slightly acidic for syrups (around 5 units of pH) the reducing nature of the samples analysed, especially in the case of honey, cannot be attributed to the high sugar content. In this line, Torto, (2009) (studying the kinetics and oxidation mechanisms of monosaccharides and some oligosaccharides) showed that reducing sugars are oxidized over the noble metal electrodes when the medium is basic; on the contrary when the pH is neutral or acidic, the oxidation process becomes undetectable. In addition, this author reported that the catalytic currents of oxidation of reducing sugars can be inhibited by the presence of the chloride anion (present in honey, although in low quantity), since this seems to deactivate the layer of gold oxide that catalyses the oxidation process of these species (Pasta, La Mantiab, & Cui, 2010).

Therefore, the discriminatory capacity of VET among honeys, syrups and adulterated honeys must be attributed to other minority antioxidant agents (polyphenols, flavonoids, carotenes, tannins, vitamin C and E, among other) that remain electroactive in the medium, which are also associated with the Faradaic current. All these agents constitute a complex mixture of characteristic antioxidants only for honeys because they are not present in adulterant syrups. The range of oxidation potentials of these compounds usually oscillates between -0.1 and -0.4 Volts (versus standard calomel electrode), which is within the working range used by VET in the present study (Bertoncelj, Dobersek, Jamnik & Golob, 2007; Peres, Sousa, Veloso, Leticia, & Días, 2016; Juan-Borrás, et al., 2017).

In addition to the implication of the role played by the Faradaic current in the discriminatory capacity of the analysed samples, the importance of non-Faradic currents must also be considered. In the specific case of honey, the latter are associated with the presence of both cationic compounds

(potassium, magnesium, iron, etc.) and inorganic anions (chlorides, nitrates, sulfates and phosphates). These currents appear in the pulse voltagrams for very short time and are directly related to the electrical conductivity of each of the studied samples (Kropf, Jamnik, Bertoncelj, & Golob, 2008). Figure 5 shows, as an example, the effect of the addition of different levels of barley syrup (Figure 5.A) or corn syrup (Fig 5.B) on the sunflower honey. Both represent the variation of the current intensity as a function of time for a potential step (400 mV) with a gold electrode. The electrochemical behaviour of adulterated sunflower honey shows an exponential decrease in the intensity as a function of time. In figure (5.A) an increase in the initial current intensity (with non-Faradic character) is observed, as increasing amounts of barley syrup are added (5, 10, 20, 40%). This is because the conductivity of this type of syrup is higher (0.55 mS/cm) than that of the honey (0.22 mS/cm). However, the value obtained from the final Faradic current at 50 ms is practically constant and equal to 48 μA in all cases.

Figure (5.B) shows that the addition of increasing amounts of corn syrup produces a progressive decrease in the initial intensity (non-Faradic current) because the conductivity of the corn syrup is much lower (0.05 mS/cm) than that of the honey (0.22 mS/cm). The initial current values show that when the amount of syrup added increases, the non-Faradic current intensity decreases (from 110 μA for pure honey to approximately 75 μA for an adulterant level of 40%). In the case of the final current (Faradic current) also undergoes a decrease in its intensity (from 48 to 35 μA for a level of adulteration of 40%). This phenomenon is not observed in the case of the barley adulterant (Fig 5.A), which maintains a practically constant level of faradic current regardless of the level of adulteration. This fact seems to indicate that the type of adulterant added can affect the electrochemical activity of the natural antioxidants contained in honey, and therefore VET could be used to detect these types of effects. Even further, this technique could help to characterize the type of adulterant added by observing the differences in the Faradic part of the pulse.

3.3. Comparison of electronic tongue and ^{1}H-NMR spectroscopy techniques by PLS analysis

To demonstrate the correlation between VET and ^{1}H-NMR analyses, the Partial Least Square (PLS) obtained by using both techniques were compared. Nine PLS prediction models were calculated taking the spectra from two honeys and each of the three adulterants for calibration (3 pairs of honeys multiplied by 3 syrups). These models were validated against the honey not considered in the couple and with the 3 syrups. PLS graphs with the prediction for corn syrup content on adulterated sunflower honey obtained from ^{1}H-NMR spectra and VET voltagrams data analyses are shown as an example in Figure 4. Measured vs. predicted values of the adulteration levels have been plotted to evaluate the performance of the created prediction linear model. As observed, the results obtained using each one of the techniques individually are quite close. Linear regression, slope and intercept are similar in both cases.

To assess the performance of the nine models for all the honeys and adulterants, Table 1 shows the PLS prediction results, number of latent variables (LV), correlation coefficient (R^2), slope, intercept, as well as RMSEP (root mean square error in the prediction) to quantitatively describe the accuracy of model outputs obtained. This table also shows the average values of these indicators. According to this information, in general terms, the performance of the models was good for both techniques, although somewhat better for NMR. The best performance was achieved by the sunflower NMR models with correlation coefficients of 0.997 (with corn), 0.996 (with barley) and 0.995 (with rice). The weakest correlation was for heather-rice (0.762) and citrus-corn (0.776) in the case of VET information. In terms of capability of prediction, models of NMR for sunflower showed a lower RMSEP, compared to the data obtained with VET, whereas in the rest of the cases the RMSEP obtained was better when VET was used for the analysis (with the only exception of heather-rice). Considering the global results for all the models with one or another technique separately, the average RMSEP achieved by ^{1}H-NMR spectroscopy was 4.174 % of error, and the RMSEP average for VET was 3.879 % of error in the prediction of the percentage of adulterant added. As a result, the capability of both techniques for this prediction was very similar.

4. Conclusions

The findings of this study confirm the validation of the VET methodology for the detection of adulteration of monofloral honey with different types of syrups, by using ^{1}H-NMR spectroscopy technique as a reference. Comparable prediction models from VET and ^{1}H-NMR spectroscopy were obtained showing a similar behaviour in predictive precision (RMSEP), with an average error less than a 5% in the estimation of adulterant content. This demonstrates similar ability in the prediction of this type of honey adulteration with a satisfactory level of error in the estimation of the percentage of added syrup. This range of precision here obtained by the VET methodology is enough for standard use as a screening technique for analysing quality control in commercial transactions of honey. Compared to ^{1}H-NMR spectroscopy, VET requires much less investment and can be implemented in portable devices, which enables the use of VET in situ. It can also be automatized in the sampling process and furthermore, the need for specialized and skilled personnel for the routine use are lower in VET than in ^{1}H-NMR spectroscopy. All these advantages reinforce VET as robust, reliable, and a relatively inexpensive technique, allowing for a more comprehensive monitoring of the adulteration of honey.

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 present work.

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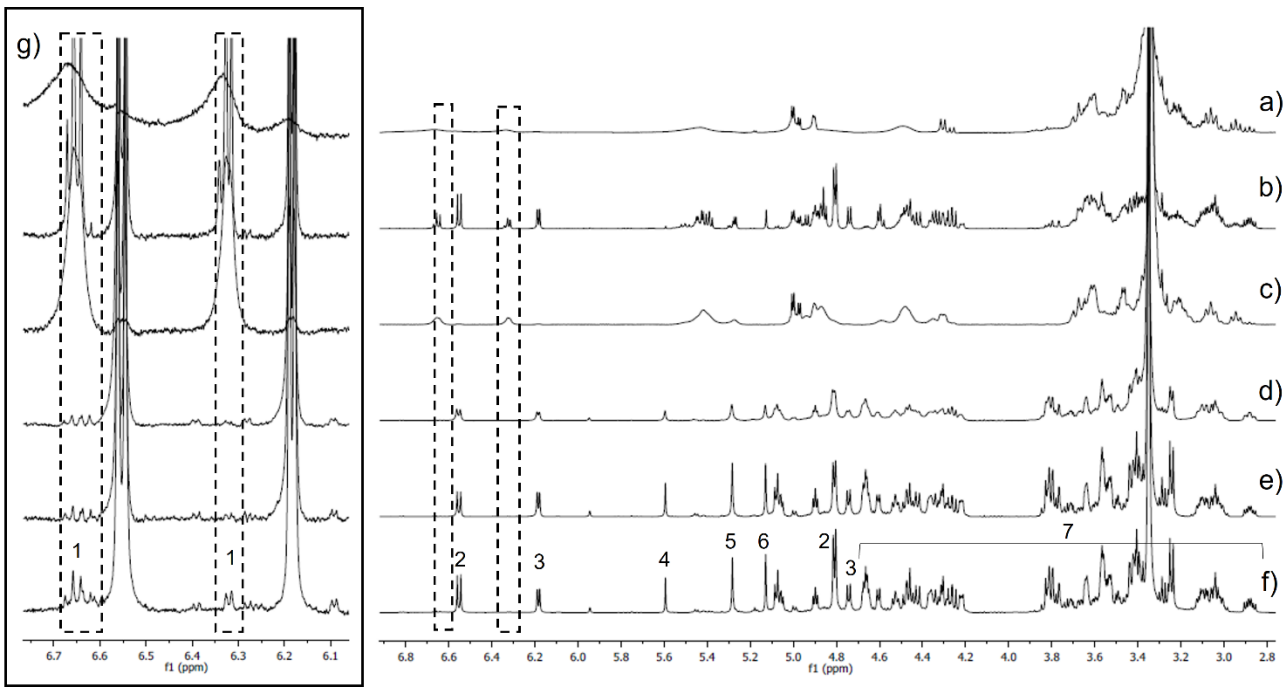


Figure 1. ^{1}H NMR spectra of pure adulterants and pure honeys in DMSO-d6: a) rice syrup, b) corn syrup, c) barley syrup, d) citrus pure honey, e) sunflower pure honey and f) heather pure honey. g) magnification from 6.0 to 6.8 ppm. Main resonances of monosaccharides are identified as follows: 1 β-maltose, 2 β-glucopyranose, 3 α-glucopyranose, 4 α-fructofuranose, 5 β-fructofuranose, 6 β-fructopyranose, 7 aliphatic protons and other oligosaccharides.

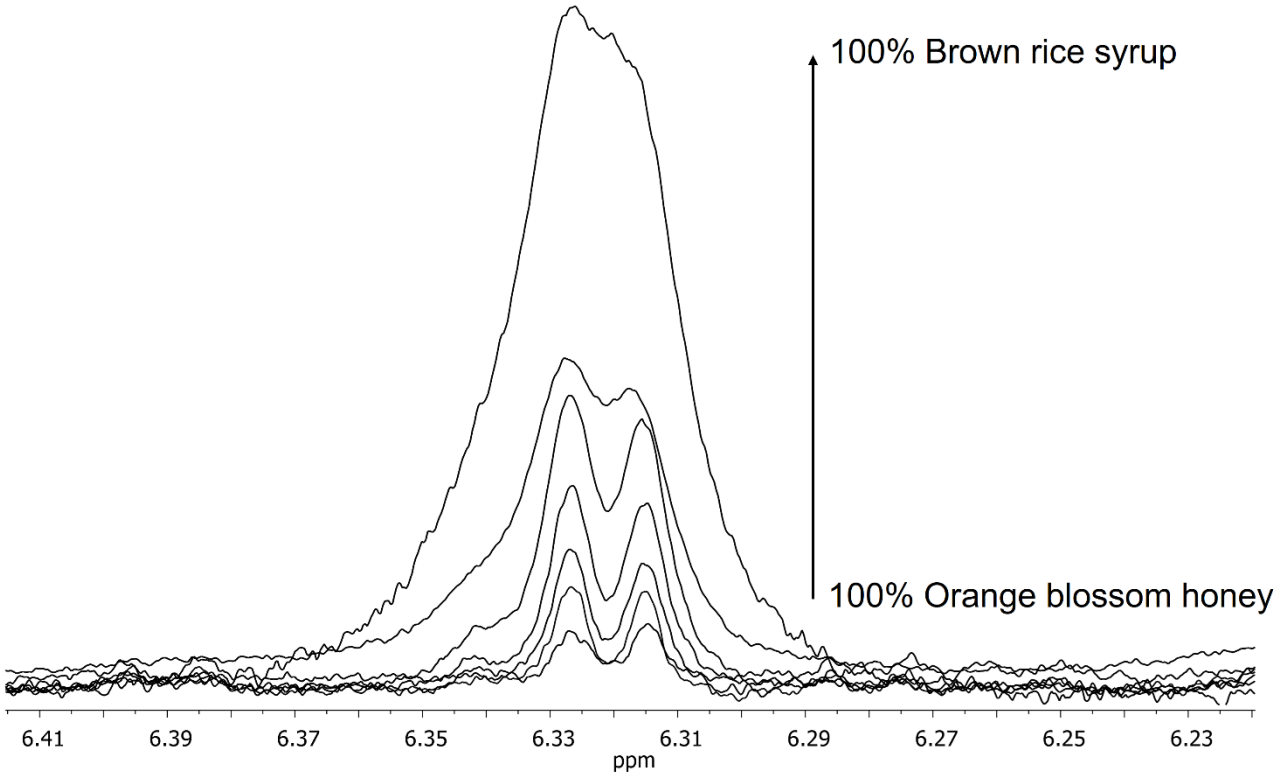


Figure 2. Expansion of spectral region for the proton linked to the anomeric carbon of β-maltose, centered at δ = 6.32 ppm, for citrus-rice adulterated honey ^{1}H NMR. . The different levels of adulteration are displayed superimposed with the same vertical scaling to show the growing intensity of the signals. From bottom to top are the spectra belong to the following samples: Citrus honey/rice syrup: 100/0, 97.5/2.5, 95/5, 90/10, 80/20, 60/40 and 0/100.

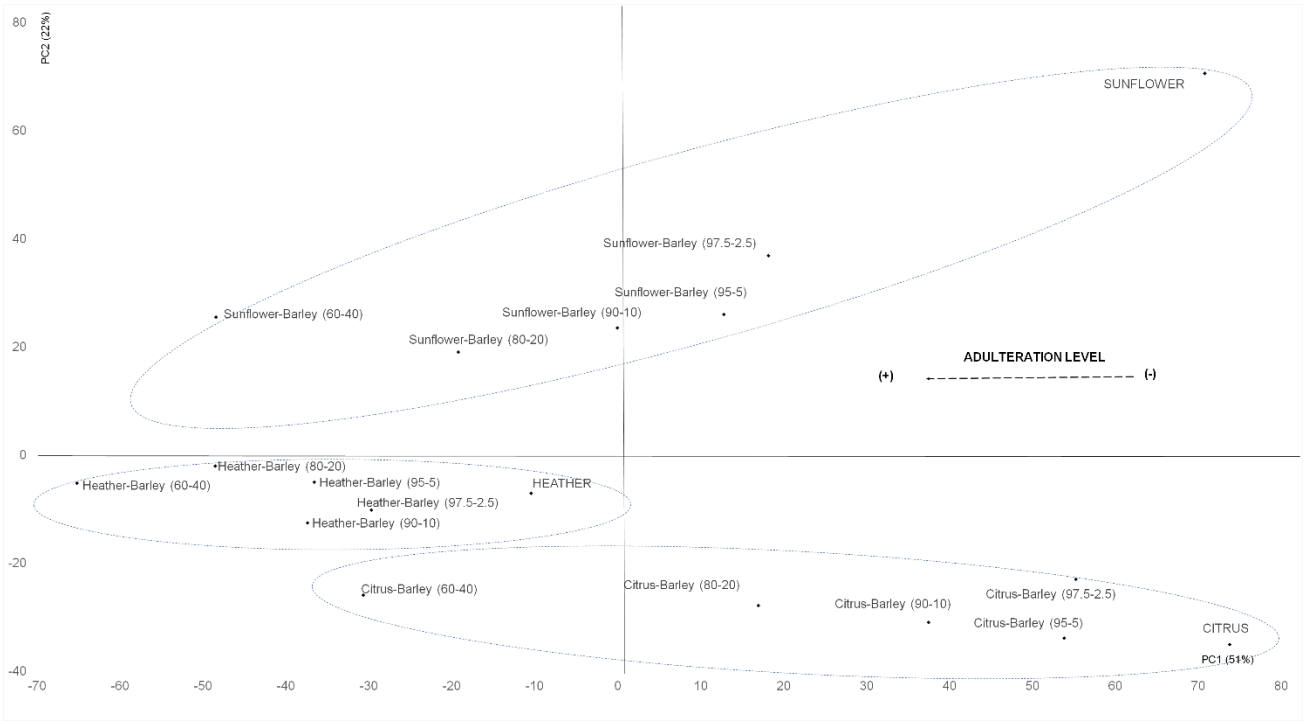
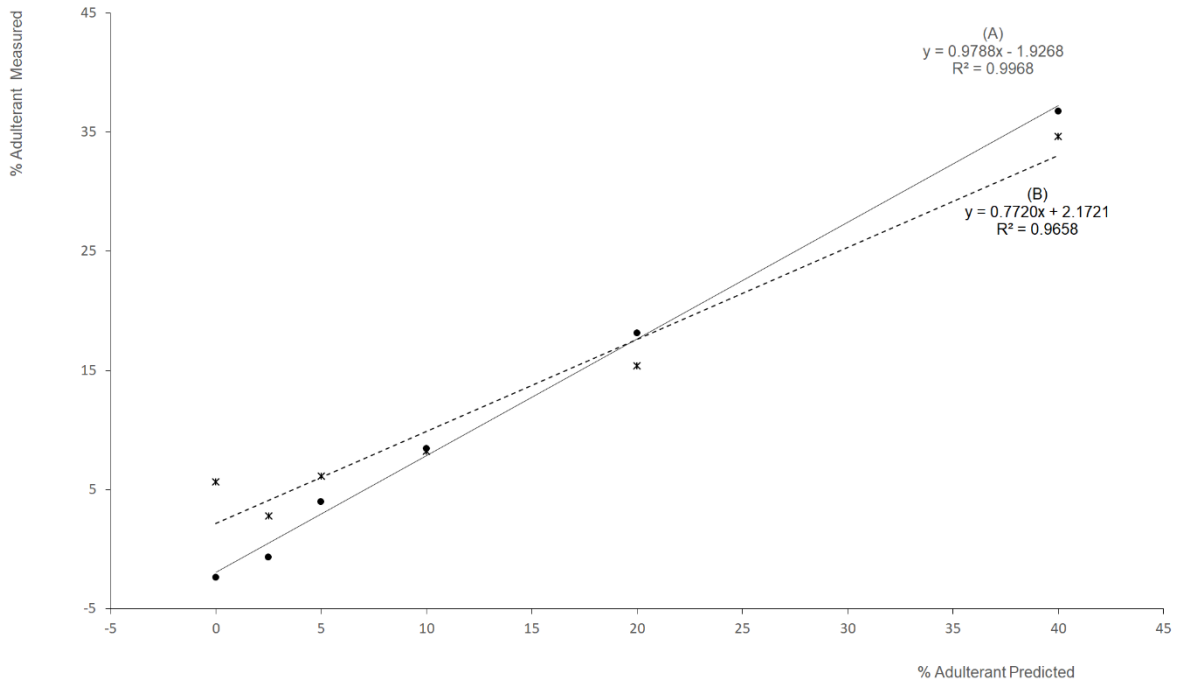


Figure 3. PCA score plot from the voltammetric electronic tongue measurements on three raw monofloral honeys samples and their adulterations at different percentages (w/w) with barley syrup (0, 2.5, 5, 10, 20, 40%)



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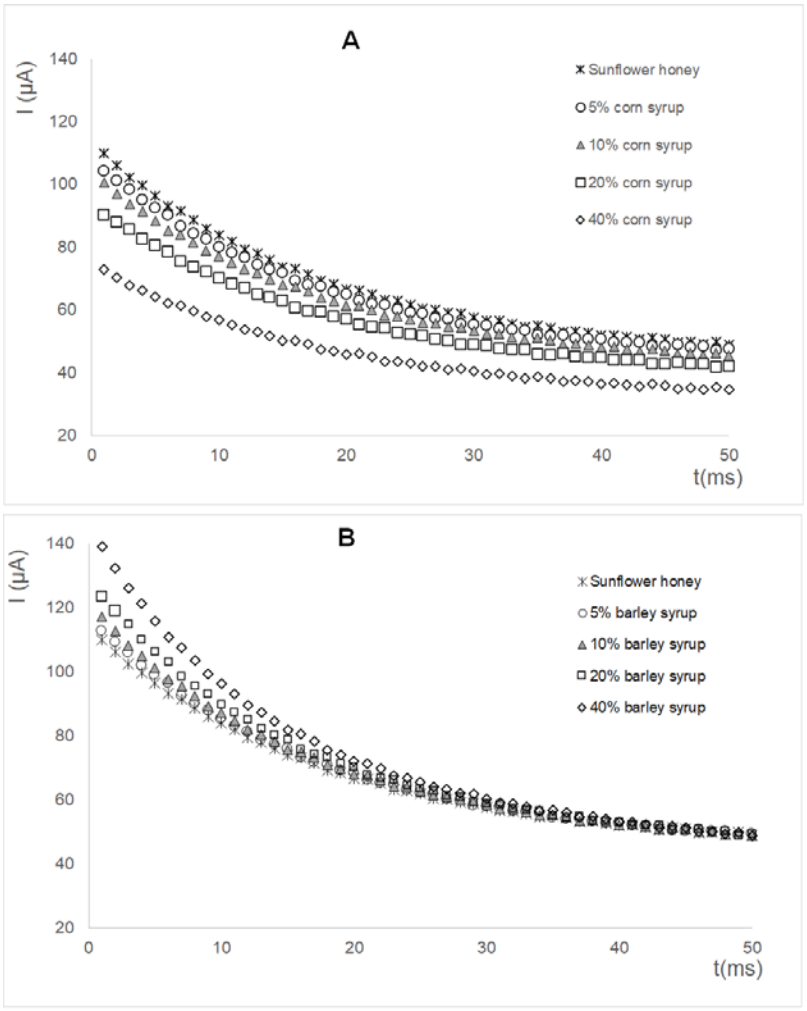
485 **Figure 4.** Measured versus predicted values of sunflower honey adulterated with corn syrup given by
486 PLS model calculated from 1H-NMR spectra data (A, continuous line) and VET voltagrams (B, dotted
487 line).



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491 **Figure 5.** Variation of the current intensity as a function of time for a potential step (400 mV with gold
492 electrode) by adding different levels of barley syrup (Figure 5.A) or corn syrup (Fig 5.B) on the
493 sunflower honey.

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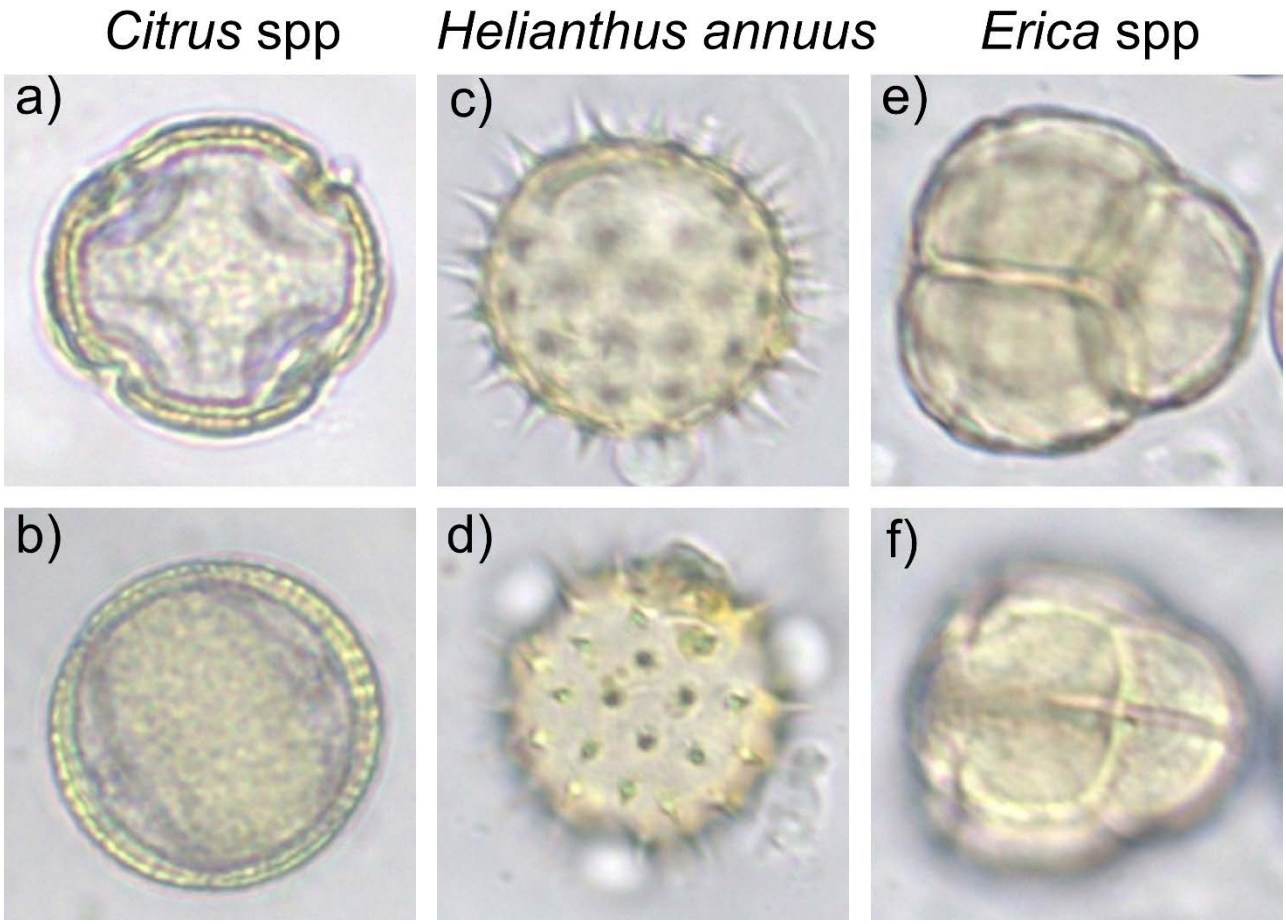

Figure S1 (Supplementary material). Photomicrographs of the 3 main pollen identified in the honey samples at 400 magnification in differential interference contrast (DIC), two different photomicrographs are shown for each type of pollen.

a) and b) images of pollen grains from *Citrus* sp. monofloral honey. The pollen analysis of three different batches revealed these contents: (Batch1*:* 16% *Citrus sp.,* 11% *Anthyllis sp.,* 12% *Brassica sp., Echium sp., Prunus dulcis, Palmaceae, Rosmarinus officinalis, Umbeliferae, Carduus Type, Helianthus annuus, Olea europaea.;* Batch2*:* 19% *Citrus sp.,* 16% *Echium sp., Brassicaceae., Hypecoum sp., Eucalyptus sp., Leguminosae, Rosmarinus officinalis, Palmaceae, Liliaceae, Olea*

europaea. Batch3*:* 12% *Citrus sp., Prunus dulcis, Rosmarinus officinalis, Brassicaceae, Taraxacum Type, Ceratonia siliqua, Asteraceae, Echium sp., Olea europaea*).

c) and d) images of pollen grains from Helianthus annuus monofloral honey. The pollen analysis of three different batches revealed these contents: (Batch1: 56% *Helianthus annuus,* 15% *Echium sp., Rubus sp., Xanthium sp., Vicia Type, Brassicaceae, Rosmarinus officinalis, Erica sp., Lavandula stoecha;* Batch2: 62*% Helianthus annuus,* 12% *Erica sp., Taraxacum type, Brassicaceae, Rubus sp., Thymus sp., Prunus dulcis;* Batch3: 48% *Helianthus annuus,* 12% *Rubus sp., Thymus sp., Onobrychis sp., Umbeliferae., Xanthium sp., Leguminosae, Centaurea cyanus, Lavandula latifolia).*

e) and f) images of pollen grains from Erica sp. monofloral honey. The pollen analysis of three different batches revealed these contents: (Batch1: 39% *Erica sp.,* 16% *Helianthus annuus, Rubus sp., Umbeliferae, Thymus sp., Prunus sp;* Batch2: 46% *Erica sp., Rubus sp., Helianthus annuus, Onobrychis sp., Brassicaceae, Thymus sp., Hypecoum sp;* Batch3*:* 40% *Erica sp.,* 15% *Echium sp., Rubus sp., Helianthus annuus, Rosmarinus officinalis, Brassicaceae, Centaurea cyanus, Hypecoum sp.).*

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529 **Table 1.** PLS prediction results obtained from the validation data for the adulteration of pure honeys (sunflower, citrus, heather) with syrup (barley, corn, rice)
530 at different percentages (40, 20, 10, 5 and 2.5% w/w) measured with VET or ^{1}H NMR. Average parameters are also shown for a global appreciation of the
531 capability of both techniques.

	LV		Correlation coeff R^2		Slope		Intercept		RMSEP	
Adulterations	VET	^{1}H NMR	VET	^{1}H NMR	VET	^{1}H NMR	VET	^{1}H NMR	VET	^{1}H NMR
Sunflower-barley	3	2	0.976	0.996	0.886	0.960	1.138	-0.655	2.276	1.521
Sunflower-corn	4	3	0.966	0.997	0.772	0.979	2.172	-1.927	3.463	2.346
Sunflower-rice	2	2	0.958	0.995	0.901	0.955	1.366	-0.172	3.232	1.364
Citrus-barely	2	1	0.967	0.957	0.899	0.956	1.977	-2.734	3.675	4.365
Citrus-corn	4	1	0.776	0.973	0.778	0.746	6.130	5.140	4.855	4.305
Citrus-rice	4	1	0.887	0.956	0.884	0.734	1.531	-0.419	4.726	5.735
Heather-barley	3	2	0.925	0.907	0.904	0.985	2.460	5.291	3.497	6.684

Heather-corn	3	4	0.922	0.962	0.939	0.873	2.974	6.814	3.889	5.961
Heather-rice	3	3	0.762	0.980	0.822	0.811	3.836	6.772	5.301	5.288
Average			0.904	0.969	0.865	0.889	2.620	2.012	3.879	4.174

Table S1. Physicochemical characterization and relative content of different pollen types present in the monofloral honeys.

Monofloral honey	**Physicochemical characterization (average)**	**Relative content of different pollen types**			
Citrus sp.		**D (>45%)**	**A (15-45%)**	**I (3-15%)**	**R (1-3%)**
Batch 1	Moisture:18.9 % Elect. Conductivity: 191 µS/cm Colour: 6.0 mm Pfund Diastase: 9.8 DN Fructosa/Glucose ratio: 1.25		16% *Citrus sp.* *35% Echium sp.* *20% Olea europaea*	11% *Anthyllis sp.,* 12% *Brassica sp.* *9% Palmaceae* *4% Prunus dulcis*	*3% Rosmarinus officinalis 3% Umbeliferae* *2% Carduus Type* *2% Helianthus annuus*
Batch 2	Moisture:17.1 % Elect. Conductivity: 195 µS/cm Colour: 10.0 mm Pfund Diastase: 8.8 DN Fructosa/Glucose ratio: 1.25		19% *Citrus sp.* 16% *Echium sp.*	*15% Brassicaceae* *15% Hypecoum sp.* *14% Eucalyptus sp.* *12% Leguminosae* *8% Olea europaea*	*2% Rosmarinus officinalis 1% Palmaceae* *1% Liliaceae*
Batch3	Moisture:18.9 % Elect. Conductivity: 185 µS/cm Colour: 5.0 mm Pfund Diastase: 7.0 DN Fructosa/Glucose ratio: 1.19		*30% Echium sp.*	*15% Brassicaceae* *15% Prunus dulcis* *12% Citrus sp.* *8% Rosmarinus officinalis* *7% Taraxacum Type* *7% Ceratonia siliqua,*	*2% Olea europaea* *2% Asteraceae*
Helianthus annuus		**D (>45%)**	**A (15-45%)**	**I (3-15%)**	**R (1-3%)**
Batch 1	Moisture:17.9 % Elect. Conductivity: 338 µS/cm	56% *Helianthus annuus,*	15% *Echium sp.*	*11% Rubus sp.* *5% Xanthium sp.*	*2% Erica sp.*

	Colour: 56.0 mm Pfund Diastase: 22.8 DN Fructosa/Glucose ratio: 1.05			*3% Vicia Type* *3% Brassicaceae*	*1% Rosmarinus officinalis 1% Lavandula stoechas*
Batch 2	Moisture:16.6 % Elect. Conductivity: 329 µS/cm Colour: 52.0 mm Pfund Diastase: 24.0 DN Fructosa/Glucose ratio: 1.00	62% *Helianthus annuus*		12% *Erica sp.* *9% Taraxacum type* *8% Brassicaceae* *5% Rubus sp.*	*1% Thymus sp.* *1% Prunus dulcis*
Batch3	Moisture:16.9 % Elect. Conductivity: 340 µS/cm Colour: 59.0 mm Pfund Diastase: 23.7 DN Fructosa/Glucose ratio: 0.99	48% *Helianthus annuus*	12% *Rubus sp.*	*8% Thymus sp.* *8% Onobrychis sp.* *6% Umbeliferae* *5% Xanthium sp.* *5% Leguminosae* *3% Centaurea cyanus*	*2% Lavandula latifolia*
Erica sp		**D (>45%)**	**A (15-45%)**	**I (3-15%)**	**R (1-3%)**
Batch 1	Moisture:17.1 % Elect. Conductivity: 510 µS/cm Colour: 88 mm Pfund Diastase: 12.8 DN Fructosa/Glucose ratio: 1.18		39% *Erica sp.* 16% *Helianthus annuus*	*14% Rubus sp.* *12% Umbeliferae* *8% Thymus sp.* *8% Prunus sp.*	*2% Asteraceae*
Batch 2	Moisture:18.0 % Elect. Conductivity: 470 µS/cm Colour: 79 mm Pfund Diastase: 13.5 DN Fructosa/Glucose ratio: 1.08	46% *Erica sp.*	*18% Rubus sp.* *15% Hypecoum sp.*	*8% Helianthus annuus* *6% Onobrychis sp.* *4% Brassicaceae*	*1% Thymus sp.*
Batch3	Moisture:17.7 % Elect. Conductivity: 489 µS/cm Colour: 83 mm Pfund Diastase: 14.1 DN Fructosa/Glucose ratio: 1.15		40% *Erica sp.* 15% *Echium sp.*	*14% Rubus sp.* *14% Helianthus annuus* *4% Ceratonia siliqua*	*3% Rosmarinus officinalis* *3% Brassicaceae* *2% Centaurea cyanus* *2% Hypecoum sp.* *2% Asteraceae*

D, predominant pollen (>45%); A, accompanying pollen (15% to 45%); I, important pollen (3% to 15%); R, minor pollen (1% to 3%).

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