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Monitoring honey adulteration with sugar syrups using an automatic pulse voltammetric electronic tongue

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ABSTRACT

The new tendency to detect adulterated honey is the development of affordable analytical equipment that is in-line and manageable, enabling rapid on site screening. Therefore, the aim of this work was to apply an electronic tongue based on potential multistep pulse voltammetry, in combination with multivariate statistical techniques to detect and quantify syrup in honey. Pure monofloral honey (heather, orange blossom and sunflower), syrup (rice, barley and corn), and samples simulating adulterated honey with different percentages of syrup (2.5, 5, 10, 20 and 40) were evaluated. An automatic, electrochemical system for cleaning and polishing the electronic tongue sensors (Ir, Rh, Pt, Au) significantly improved the repeatability and accuracy of the measurements. PCA analysis showed that the proposed methodology is able to distinguish between types of pure honey and syrup, and their different levels of adulterants. A subsequent PLS analysis successfully predicted the level of the

adulterants in each honey, achieving good correlations considering the adjusting parameters. The best results being for sunflower honey adulterated with corn syrup and heather honey with barley syrup (r^2=0.997), and heather with corn (r^2=0.994) whereas the weakest was found for heather honey adulterated with brown rice syrup (r^2=0.763) and orange blossom honey with corn syrup (r^2=0.879). The measurement system here proposed could be a very quick and effective option for the honey packaging sector with the finality of providing information about a characteristic as important as the adulteration of honey.

Keywords: honey adulteration, syrups, pulse voltammetry, electronic tongue

1. Introduction

Honey is a nutritional natural sweetener highly valued for its healing properties (Naila et al., 2018, Cabanero et al., 2006; Padovan et al., 2003; Ruiz-Matute et al., 2010; Bázár et al., 2016). The European Commission has stipulated that nothing should be added to honey (European Commission, 2002), but the limited availability and its price have provided major incentives for adulteration (Anklam, 1998). Honey is adulterated mainly with cheaper sweeteners such as sugar syrups that simulate its own sugar composition (Naila et al., 2018, Li et al., 2017; Sobrino-Gregorio et al., 2017, Cai et al., 2013). Adulterated honey affects the international honey market and the economy of the producers. In addition, it could have negative effects on consumer's nutrition and health (Tosun, 2013). Therefore, guaranteeing the authenticity of honey has become a very important issue for everyone involved in the food chain (Sobrino-Gregorio, 2017).

In recent years, a large number of analytical methods have been used to differentiate genuine honey from adulterated. Among them, the most recognized are NMR spectroscopy (Bertelli et al., 2010; Boffo et al., 2012; Davide and Massimo, 2010; Ohmenhaeuser et al., 2013; De Oliveira et al., 2014) and stable carbon isotopic ratio

mass spectrometry (SCIRA) (Elfleing & Raezke, 2008; Adnan et al., 2012; Simsek et al., 2012; Tosun, 2013). Another commonly used method is the reflectance-Fourier transforms infrared spectroscopy (Oroian & Ropciuc, 2017, Rios-Corripio et al., 2012;), high performance liquid chromatography (HPLC) to detect starch syrups (Wang et al., 2015), enzymatic activity (diastase, invertase) (Serra et al., 2000), specific markers (Xue et al., 2013) and differential scanning calorimetry (DSC) (Cordella et al., 2002 y 2003; Sobrino-Gregorio et al., 2017).

However, using these techniques individually the results obtained are not always conclusive, therefore, to guarantee the purity in honey the combination of several of them is required. Moreover, these techniques are very expensive, they require highly specialized equipment and are time-consuming (Sobrino-Gregorio et al., 2017).

To identify the authenticity of honey the industry needs to have simple, fast and easy to handle techniques without the need for expensive equipment and highly skilled workers (Bougrini et al., 2016; Juan-Borrás et al., 2017). Furthermore, the honey sector does not require data of exact levels of adulteration of honey, since any type of addition is prohibited. Only with a screening technique that able is to detect the slightest adulteration is enough.

Among the most promising techniques that fulfill this requirement, in addition to being more environmentally friendly than the usual methods, the electronic tongue has the advantage, as it can be an alternative tool to the traditional analytic methods (Bougrini et al., 2016). Unlike the traditional methods, electronic tongues do not obtain information about the nature of the compounds under consideration, but only present a digital fingerprint of the food material (Ghasemi-Varnamkhasti et al., 2010). It is also a qualitative analytical technique that permits recognition, classification or identification

of samples, depending on the composition of the sensor array and the mathematical procedure adopted for data treatment.

Electronic tongue systems are based on an array of sensors with low selectivity while being sensitive to several components in the measured sample (cross-sensitivity). The signals collected by these sensors are processed by means of pattern recognition tools in order to generate prediction models that allow the classification of the samples and the quantification of some of their physicochemical properties (Gutés et al., 2007).

There are several alternatives to electronic tongue systems, the voltammetric being one of the most used (Martínez-Mañez et al., 2005; Lvova et al., 2006; Winquist et al., 2005), which has different advantages: high sensitivity, versatility, simplicity, robustness and good signal to noise ratio (Winquisk, 2008).

These techniques, using arrays of electrodes, is at present the most popular for the design of electronic tongue systems, which include linear voltammetry, differential pulse voltammetry (Bataller et al., 2013), stripping voltammetry and over all cyclic voltammetry (Campos et al., 2010). Cyclic voltammetry is the most widely used technique (Bollo et al., 2004; De Beer et al., 2004; Dogan et al., 2005) and the obtained voltammogram permits the characterisation of electrochemical processes (oxidation–reduction) over a wide potential range. On the other hand, pulse voltammetry is used when higher sensitivity and resolution are required, allowing the detection of lower concentrations of compounds (Escobar et al., 2013). In all cases the enormous amount of data generated by these systems must be processed using appropriate multivariate analysis techniques such as PCA (principal component analysis), LDA (linear discriminant analysis) or CA (cluster analysis) (Benedetti et al., 2004; Dias et al., 2008; Wei et al., 2009).

Electronic tongue systems are capable of identifying and classifying liquid samples such as wine, beer, coffee, milk, juices, teas and vegetable oils (Schreyer & Mikkelsen, 2000; Parra et al., 2004; Apetrei et al., 2005; Huang et al., 2007; Chen et al., 2008; Moreno-Codinachs et al., 2008; Rodríguez-Méndez et al., 2008; He et al., 2009; Oliveri et al., 2009; Gutiérrez et al., 2010; Gutierrez-Capitan et al., 2013; Apetrei & Apetrei, 2014; Veloso et al., 2016). Moreover, it is used in quality assessment of solid foods such as meat, fish, fruit and vegetables (Han et al., 2008; Rodríguez-Méndez et al., 2009; Campos et al., 2010; Labrador et al., 2010). In the context of discriminating different classes within the same food type, it has been successfully used in honey, specifically focused on its differentiation according to its botanical and geographical origin (Dias et al., 2008; Wei et al., 2009; Wei & Wang, 2011; Major et al., 2011; Escriche et al., 2012; Garcia-Breijo et al., 2013; Tiwari et al., 2013; Sousa et al., 2014; Bougrini et al., 2016; Juan-Borrás et al., 2017).

One of the most promising applications of the electronic tongue systems is the detection of food adulterations. Good results have been reported in the identification of sunflower oil introduced in argan oil (Bougrini et al., 2014) or in the case of goat milk adulterated with bovine milk (Dias et al., 2009). However, little research about the use of electronic tongue systems to identify adulteration of honey has been reported using pulse voltammetry (Men et al., 2014) or cyclic voltammetry (Bougrini et al., 2016; Ropciuc et al., 2017). However, the application of pulse voltammetry, in the above context, could provide important advantages since it has higher sensitivity and resolution (Bataller et al., 2013).

Nevertheless, an important problem that limits the use of electronic tongues as a technique for on-line quality controls is that of sensor system cleaning. In the aim to solve this, a mechanical system was developed by Swedish Sensor Center for polishing

123 the electrodes of the electronic tongues (Olsson et al., 2006). However, it significantly
124 limits the utility of the technique in controlling automated processes, as it requires high
125 maintenance and costs. As a cheaper and easier alternative, the same group proposed the
126 use of electropolishing to clean the electronic tongues (Holmin et al., 2004). The
127 procedure consists of applying a high enough voltage to oxidize the surface of the
128 electrodes, and to apply proper cathodic voltage to regenerate the different metal
129 surfaces. Although studies using this technique were promising, the methodology was
130 not optimized for systems that have high concentrations or high levels of contaminant
131 load. Honey falls within the group of substances for which there is no well-defined
132 electropolishing methodology.

133 Taking this into consideration, the aim of this study was to optimize an adequate
134 electropolishing system to investigate the capacity of a pulse voltammetric electronic
135 tongue, which consisted of a set of metal electrodes, to differentiate the presence of
136 syrups in honey samples simulating various levels of adulteration.

137 **2. Materials and methods**

138 **2.1. Samples preparation**

139 Three types of raw honey harvested in 2016, provided by the company Melazahar
140 (Montroy, Valencia), were used in this study: sunflower (*Helianthus annuus*); orange
141 blossom (*Citrus spp*.) and heather (*Erica spp*.). They were selected based on their
142 different physicochemical characteristics (Juan-Borrás et al., 2015). The botanical
143 categorization of all the batches was carried out by means of pollinic analysis following
144 the recommendations of the International Commission for Bee Botany (Von Der Ohe et
145 al., 2004). Microscopic examination, identification and the interpretation of pollen types
146 were carried out by an experienced pollen analyst, using pollen slides and references
147 (Sáenz & Gómez, 2000; Persano-Oddo & Piro, 2004). Furthermore, three kinds of

syrups from different origins were used: barley (La Finestra sul Cielo, Italy); corn (Roquette Laissa, Spain) and brown rice (Mitoku Macrobiotic, Japan).

The samples evaluated in the present work were: three pure syrups, three pure honeys and a mixture of both in different percentages (40, 20, 10, 5 and 2.5%, respectively) simulating the adulteration of honey. In each case, 8 g of sample (considered on a dry basis) were used. For this, the moisture content was obtained by using a refractometer (Abbe-type model T1; Atago, Bellevue, WA, USA) and the Chataway tables in accordance with the Harmonized Methods of the European Honey Commission (Bogdanov, 2009). All samples were analysed three times achieving four repetitions for each replication.

2.2. Equipment

The measuring equipment is based on a potentiostat designed in the Institute of Molecular Recognition and Technological Development (IDM) at the Universitat Politècnica de València (Campos et al., 2013). This device allows performing pulse voltammetry measurements where the potentials and lengths of the pulses can be configured for each specific application. In this particular work, 40 pulses of 50 ms are applied. The voltages distribution is similar to a stair case voltammetry in increasing (or decreasing) steps of 200 mV between +1 V and -1 V (to avoid water electrolysis), and the potential is set to zero after each increment (Figure 1).

The voltammetry tests are measured with four working electrodes (Ir, Rh, Pt and Au) housed inside a stainless-steel cylinder used as the electronic tongue body. A stainless steel circular piece is used as counter electrode and a calomel electrode is used as reference.

An integrated system of solenoid valves and a pump permit the automatic injection of liquid samples into a specifically designed measurement chamber. This complete system allows the implementation of an innovative electrochemical polishing of the working electrodes. For each metal, a configuration of basic or acidic solution is used when a sequence of cathodic and anodic pulse (or reversed) is applied to them. The aim is desorbing the organic material accumulated at the surface of the electrodes, and detaching any oxide layer that may have been formed (Table 1).

An in-house design of a specific software manages both the measuring equipment and the pumping system. It performs a complete set of measurements with the same setup, and stors the results for a later statistical analysis.

This system was patented in 2016, under the name “Sistema y método de control de la calidad del agua en plantas de tratamiento”, which translation would be “System and method to control water quality in treatment plants”, property of the company Fomento Agrícola Castellonense, S.A. and the by the Interuniversity Research Institute for Molecular Recognition and Technological Development (IDM) of the Universitat Politècnica de València, with reference number P201631405 (Bataller et al., 2016).

2.3. Statistical analysis

Multivariate statistical analysis techniques were used to analyse the data gathered for this study. Principal Components Analysis (PCA) was used to discriminate between samples and Partial Least Square (PLS) to quantify the content of honey adulterant in the analysed samples. The PLS model was calibrated with 66% of the data set and validated with the remaining 33%. Model’s assessment is done by comparing real versus predicted adulteration levels. The parameters used are 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 (Bataller et al., 2012).

All these statistical studies have been performed with Solo 8.6 software (Eigenvector Research, Inc., Wenatchee, Washington, DC, USA).

3. Results and discussion

3.1. Differentiation of pure honeys and syrups

A PCA analysis was applied (from the data generated by the four electrodes of the electronic tongue) in order to show if there was a classification of the different types of pure samples (honeys and syrups). Figure 2 shows the score plot of this analysis, in which the first two principal components together explain 80.01% of the data variability, specifically 59.59% by PC1 and 20.42% by PC2. Discrimination between honeys and syrups is mainly determined by the X axis (PC1), where the honey samples are in the centre of the score graph and the syrups are placed on both sides (on the left the barley and brown rice syrups, on the right the corn). Since, proximity between samples indicates similar behaviour in terms of the electrochemical response of the sensors, small differences between barley and brown rice syrups with respect to corn syrups were found. On the contrary, the type of honey is differentiated by PC2, where heather honey is in the upper half and sunflower honey in the lower, whereas orange blossom honey is in the middle.

Once proven that this methodology could differentiate between all types of pure samples analysed, the next phase was to verify if this type of electronic tongue was able to discriminate honeys in which syrups have been added.

3.2. Differentiation by adulteration levels

Figure 3 shows, as an example, the behaviour of the signal obtained by applying the corresponding potential pulse pattern to sunflower honey adulterated with barley syrup. There is a clear differentiation between the signals obtained for syrup, pure honey and the different percentages of adulteration (40, 20, 10, 5 and 2.5%, respectively). It is evident that the signals are affected by the samples and the adulteration levels. The highest signal corresponds to pure syrup which progressively decreases to pure honey, going through its different and ordered stages of adulteration. This behaviour was in most cases constant, regardless of the type of syrup and honey.

For the purpose of evaluating from a descriptive point of view, the global effect of the electronic tongue response in the pure honeys, syrups and their corresponding adulteration levels, different Principal Component Analysis (PCA) were carried out. Figure 4 shows, as an example, the PCA performed in the case of adding barley syrup (40, 20, 10, 5 and 2.5%) to the three pure honeys. In this figure (4.A to 4.C), the two principal components represent 86.5% (PC1: 52.50%; PC2: 34.02%); 83.98% (PC1: 67.41%; PC2: 16.57%) and 78.87% (PC1: 61.89%; PC2:19.98%) for sunflower; orange blossom and heather, respectively. Pure barley syrup and honey with 40% of barley syrup are in all cases on the left side of the plots (but in opposite quadrants), whereas the rest of the samples are placed on the right side. In all these PCA plots, a progressive, ordered and clear tendency is observed in relation to the adulteration level. The higher level (40%) is farther away from the pure honey, whereas the lower level (2.5%) is closer to it.

Similar results were reported by Bougrini et al., (2016) using cyclic voltammetry for adulteration detection, from 2 to 20%, of pure honey although glucose and sacharose syrups were added. The values of adulteration detected by these authors in honey are even better than those described by them in the case of adulteration in argan oil

adulterated with different proportions of sunflower oil (10 to 70%) (Bougrini et al., 2014). Ropciuc et al. (2017), using cyclic voltammetry (with Ag and Au as working electrodes) differentiated honeys adulterated with inverted sugar and malt wort only when they did not exceed 20%.

The present work confirms that using pulse voltammetry allowed for further possibilities by designing a specific pulse pattern. In addition to the automation of the electrochemical cleaning process while providing good reproducibility of the sensors and good classification results, it also permitted the detection of a wider adulteration range (up to 40%). Moreover, among other advantages it was observed that the time required to analyse one sample using the pulse voltammetry technique is considerably less: 8 seconds to scan with the 4 electrodes; 40 seconds for 5 iterations; 12 seconds for electropolishing per electrode; 4 minutes for the final cleaning of the sensor system if 5 consecutive cleanings are performed. However, considering the protocol described by Bougrini et al. in 2016, the cleaning alone (disassembled, manual cleaning of electrodes, electrochemical cell re-assembled, etc.) takes at least 20 min.

3.3. PLS analysis: correlation of pulse voltammetric data with the level of adulteration

In order to verify whether the data provided by the electronic tongue could be useful in predicting the adulteration of pure honeys (sunflower, orange blossom, heather) with syrup (barley, corn, brown rice) at different percentages (40, 20, 10, 5 and 2.5%), a Partial Least Square (PLS) analysis was applied. Nine PLS models of prediction were created (3 honeys multiplied by 3 syrups) with the voltammetric experimental data obtained from the four metallic electrodes (Ir, Rh, Pt, Au). Figure 5 shows one of these PLS graphs (heather honey adulterated with barley syrup) in which measured vs. predicted values of the adulteration levels are plotted together in order to evaluate the

performance of the created prediction linear model. Table 2 shows the PLS prediction results (slope, intercept, the regression coefficient, number of latent variables and RMSEP) for the nine models obtained. In most cases, there is a good result, the best one being for sunflower-corn and heather-barley with correlation coefficients of 0.997 and heather-corn with 0.994. The weakest correlation was for heather-brown rice (0.763) and orange blossom-corn (0.879). In order to quantitatively describe the accuracy of model outputs obtained, the RMSEP were shown (Table 2). The best model in terms of capability of prediction corresponded to that obtained for heather-barley (0.834) followed by sunflower- barley (1.252). The worst model was for orange blossom-corn (5.261) and header brown-rice (5.159).

Cai, et al., in 2013, applied cyclic voltammetry in Chinese Angelica honey adulterated with rice syrup (from 20% to 50%). They were able to prove that in quantitative analysis of honey adulteration, a multiple linear regression (MLR) model fitted and predicted well with the square of the correlation coefficients (Rc=0.921 and Rp=0.898). Other authors proposed the combination of PLS with Fuzzy ARTMAP tools to improve the classification of honey adulterated in different proportions (from 0 to 70%) when a voltammetric electronic tongue system is applied (Men et al, 2014).

4. Conclusions

This paper has presented for the first time that an innovative automatic pulse voltammetry can be applied to quantify the presence of syrups in honey. The outcome is the possibility that this type of electronic tongue (with automatic, electrochemical system for cleaning and polishing the electronic tongue sensors) permits detecting this kind of fraud in a bee product to which no addition of substances is allowed. PCA analysis demonstrated that this automatic pulse voltammetry electronic tongue system, made of four metallic electrodes (Ir, Rh, Pt, Au) was capable of not only differentiating

between types of pure honey and pure syrups but also to discriminate honeys to which syrups have been added at different levels. The PLS models are capable of predicting the additions of adulterants in different types of honey, and therefore provides a powerful tool to quantify their level of incorporation.

The present findings might help to solve the necessity to have a manageable and in-line analytical equipment that enables rapid on site screening and also more affordable for the apiculture sector. However, future studies on the current topic are recommended in order to create a wide and comprehensive data base of pure types of honey from different botanical and geographical origin.

Conflicts of interest

The authors declare that they have no conflict of interest.

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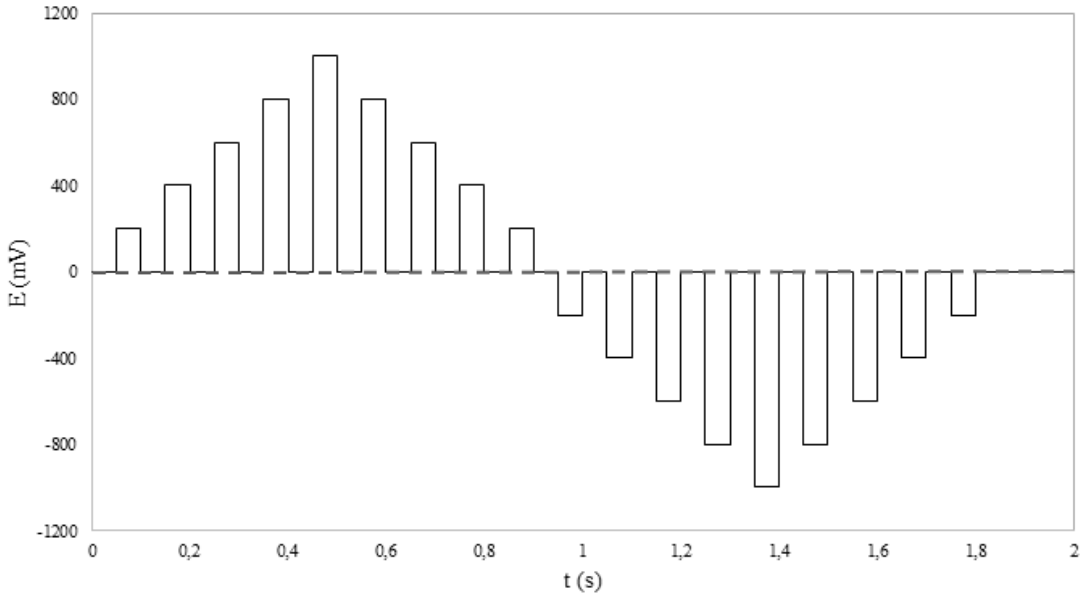
552

553 **Figure caption**

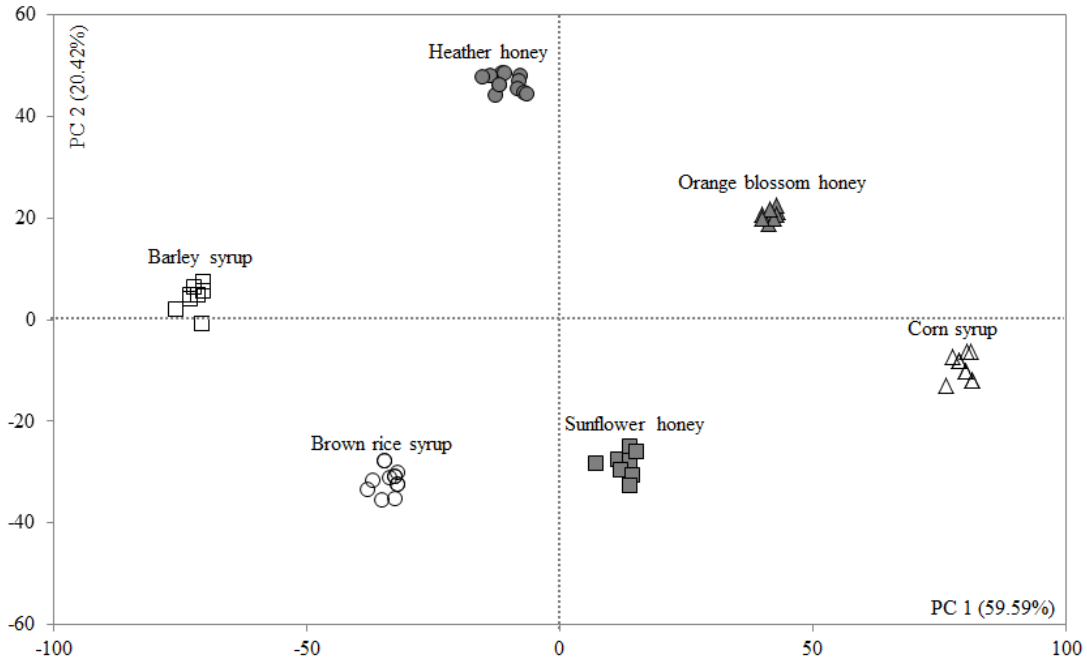
554 **Figure 1**. Voltammetric pulse pattern.



555

556 **Figure 2**. Score plot of the PCA performed on pure honeys (sunflower, orange blossom,

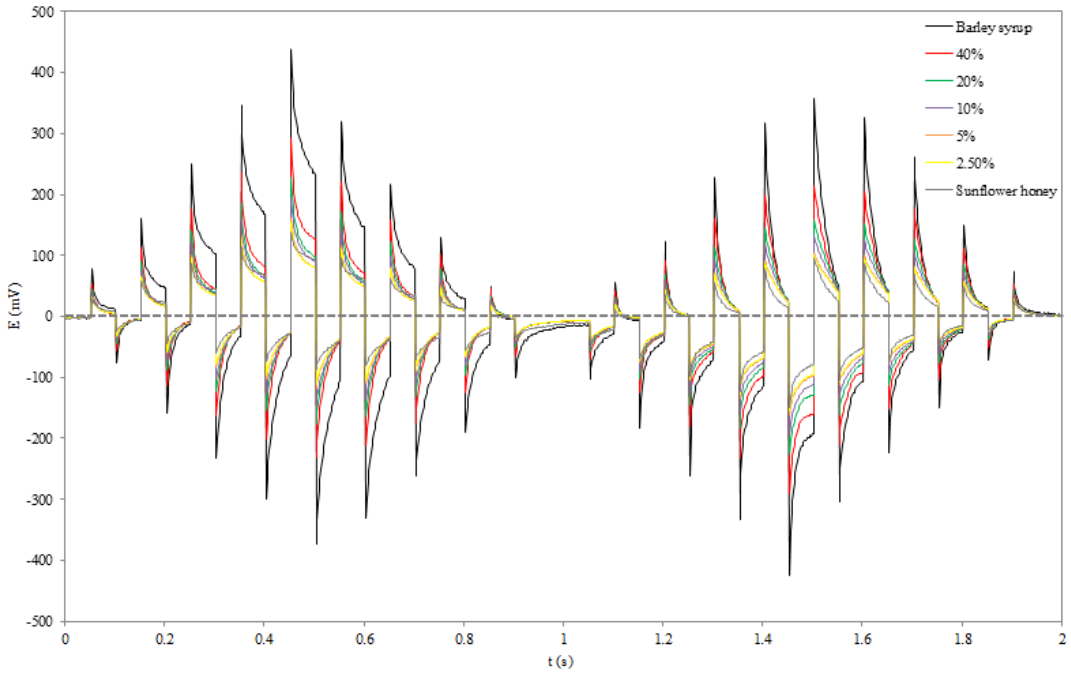
557 heather) and pure syrups (barley, corn, brown rice) samples.



558

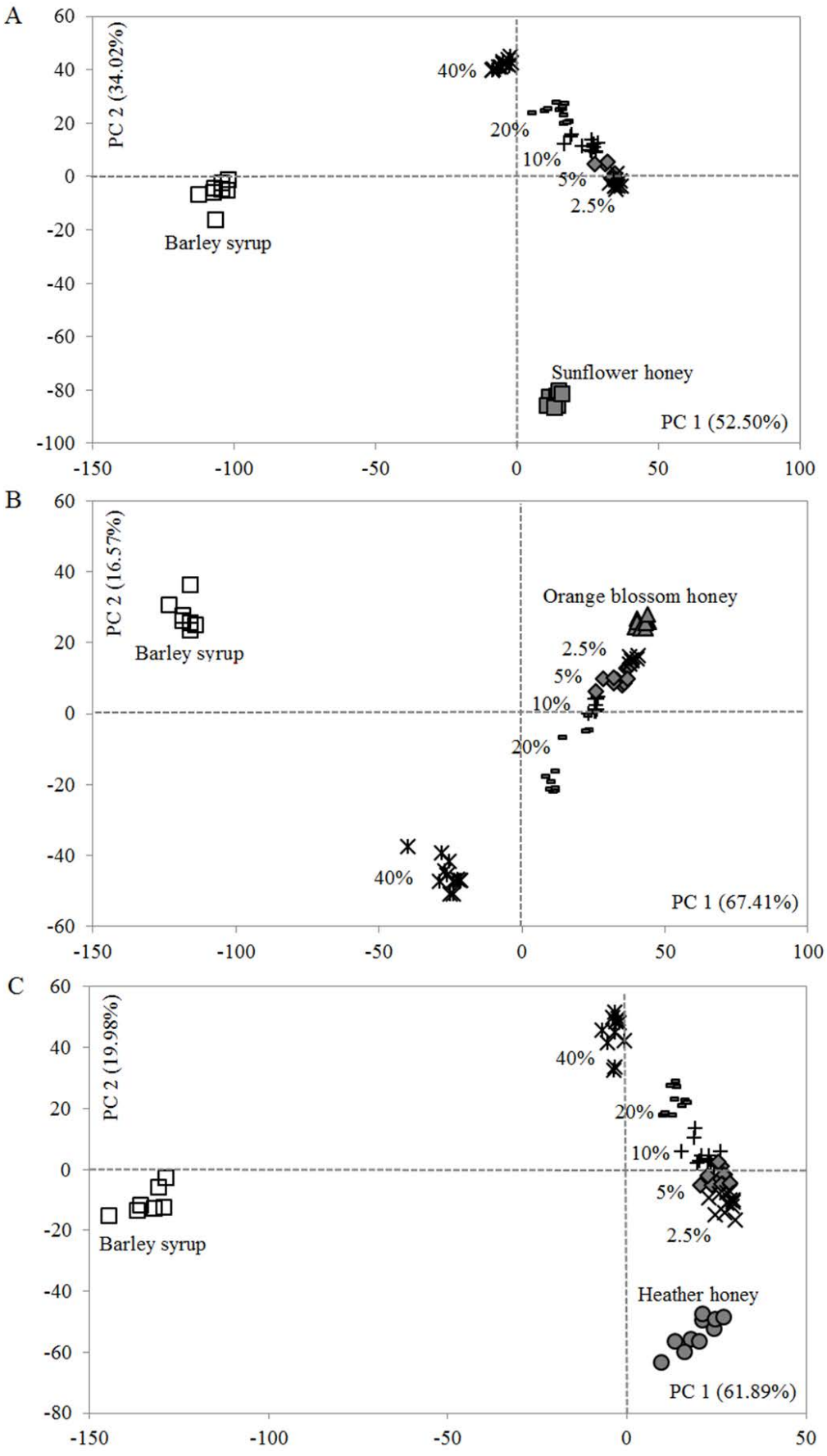
559 **Figure 3**. Electrochemical trace of the layered sequence of the potential for sunflower

560 honey adulterated with barley syrup at different levels (40, 20, 10, 5 and 2.5%).



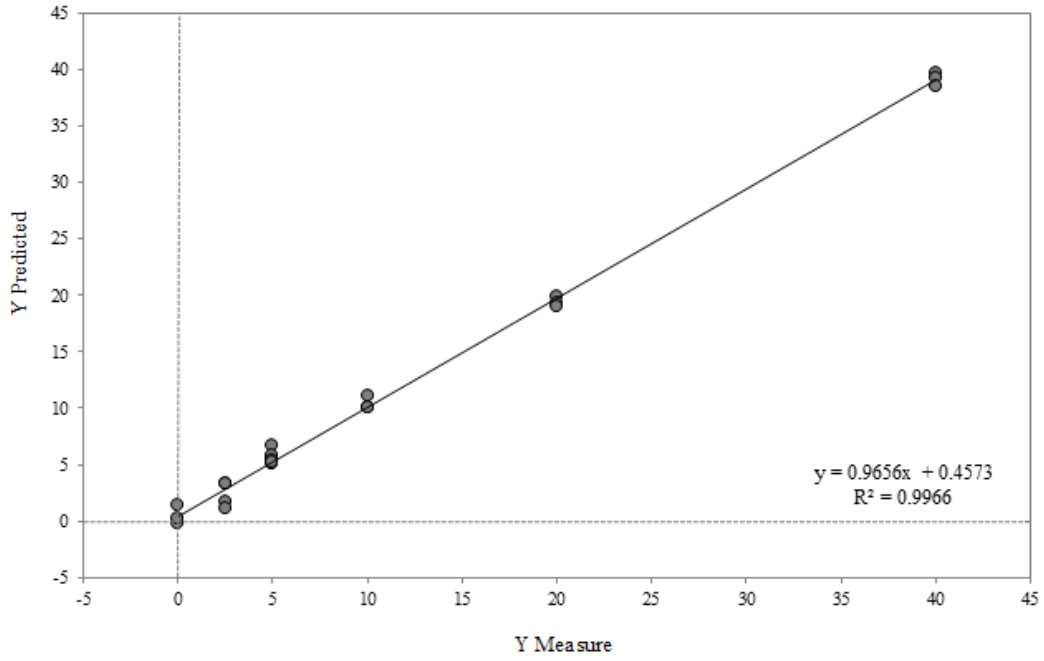
561

562 **Figure 4**. Scores plot of the PCA performed on barley syrup and pure honey (A:
563 sunflower; B: orange blossom; C: heather hone), and mixtures of both in different
564 percentages (40, 20, 10, 5 and 2.5 %) simulating the adulteration of honey.


A
PC 2 (34.02%)
PC 1 (52.50%)
40%
20%
10%
5%
2.5%
Barley syrup
Sunflower honey
B
PC 2 (16.57%)
PC 1 (67.41%)
Barley syrup
Orange blossom honey
2.5%
5%
10%
20%
40%
C
PC 2 (19.98%)
PC 1 (61.89%)
40%
20%
10%
5%
2.5%
Barley syrup
Heather honey

565

566 **Figure 5**. Predicted versus measured values of heather honey adulterated with barley
567 syrup given by PLS model.



568

569 **Highlights**

570 -Pulse voltammetry can be useful in detecting and quantifying syrups in honey

571 -This methodology can distinguish between pure honeys and syrups

572 -This methodology can distinguish among different levels of adulterants

573 -PLS analysis can predict the level of adulteration with syrups in honeys

574 Table 1. Electrochemical polishing of the working electrodes: configuration of basic or
575 acidic solution in the sequence of cathodic and anodic pulse applied to the different
576 metals.

Electrodes	Cathodic pulse (mV)	Anodic pulse (mV)	Rest pulse (mV)	Polishing media
Ir	+900	-500	0	Acidic

Rh	+1500	-1500	0	Basic
Pt	+1800	-500	0	Acidic
Au	+1500	-1500	0	Basic

577

578 **Table 2.** PLS prediction results obtained from the validation data for the adulteration of
579 pure honeys (sunflower, orange blossom, heather) with syrup (barley, corn, brown rice)
580 at different percentages (40, 20, 10, 5 and 2.5%).

581

Adulterations	No. latent variables	Correlation coefficient	Slope	Intercept	RMSEP
Sunflower-barley	4	0.991	0.999	0.206	1.252
Sunflower-corn	5	0.997	0.937	1.858	2.622
Sunflower-brown rice	2	0.949	0.909	1.073	3.489
Orange blossom- barley	7	0.993	0.983	0.589	1.336
Orange blossom-corn	6	0.879	0.847	1.234	5.261
Orange blossom- brown rice	6	0.988	1.029	0.203	1.681
Heather- barley	5	0.997	0.966	0.457	0.834
Heather- corn	5	0.994	1.012	0.997	1.479
Heather- brown rice	4	0.763	0.823	3.936	5.159

582