COMPARISON OF E-NOSES: THE CASE STUDY OF HONEY

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ABSTRACT

Authentication is a major research theme in food analysis. Electronic noses (e-Noses) represent an effective tool for food authentication and - among others - for the determination of food origin. In this work we compare the performance of two e-Noses (metal-oxide-sensor based *vs* mass-spectrometry based) in the determination of geographic origin of honey. We analyzed 14 honey samples from South Tyrol, an Italian alpine region, which were compared with other 12 commercial samples from diverse European origins. Both e-Noses afforded 85% of correct identifications. Mass spectrometry provided a deep analytical insight, thanks to the possibility to determine mass peaks with good accuracy.

Keywords: South Tyrol Italian honeys, geographic origin, PTR-ToF-MS, e-Nose

1. INTRODUCTION

Gas-chromatographic methods represent the benchmark for food volatile analysis. In spite of its robustness and analytical power, gas-chromatographic analysis, being a separation-based technique, is time-consuming and has a low analytical throughput. An alternative analytical approach is based on the employment of electronic Noses (e-Noses). In the e-Nose, an array of electrochemical sensors (GARDNER, 1999) or a mass spectrometer (PÉREZ PAVÓN *et al*., 2006) provide a fingerprint of the headspace of a given sample. Typically an e-Nose, trained using samples of known origin, can be employed to recognize and predict sample identity on the basis of a specific fingerprint. Unlike GC-MS, the e-Nose provides little information as to the actual composition of the sample headspace; on the other hand e-Noses are generally easy to use, they provide a high analytical throughput and they are relatively inexpensive.

Proton Transfer Reaction-Mass Spectrometry (PTR-MS), similarly to an MS-based e-Nose, performs a rapid and direct analysis of the headspace of the sample. Unlike in MS-based e-Noses the use of a *soft* ionization approach allows to minimize fragmentation, thus increasing the informational content of the mass spectral fingerprint (Hansel *et al*., 1995). The coupling of PTR-MS to Time-of-Flight (ToF) mass analyzers has further enhanced the performance of the technique, allowing for high mass and time resolution. PTR-ToF-MS has already been employed in the determination of food origin, with applications, among others, on cheese (GALLE *et al*., 2011), ham (DEL PULGAR *et al*., 2011) and coffee (YENER *et al*., 2015).

Honey is traditionally consumed and appreciated worldwide, mainly because of its organoleptic properties and nutritional value. Even though the main constituents of honey are sugar and water, a great variety of aroma compounds can also be encountered.

Gas Chromatography-Mass Spectrometry (GC-MS) was often employed to describe the composition of honey headspace, and up to 400 distinct VOCs were reported is a single honey type (GUYOT *et al*., 1998). The two main factors affecting the quality of honey are its botanical and geographical origin. The botanical origin of the nectar and plant secretions is a major source of aroma compounds and aroma precursors but geographical origin, through the influence of soil and climate, also plays an important role. Several GC-MS studies were carried out with the aim to study and predict the botanical and/or geographical origin of honeys (CUEVAS-GLORY *et al*., 2007; KAŠKONIENĖ and VENSKUTONIS, 2010). This approach mostly applies in the case of honeys deriving from a single plant species (*unifloral* honeys) and researchers have claimed the discovery of plant specific-markers (CASTRO-VÁZQUEZ *et al*., 2006; JERKOVIĆ *et al*., 2006; VERZERA *et al*., 2014; JERKOVIĆ and KUŚ, 2014); these most often include terpenes, norisoprenoids, nitriles or phenolic compounds and their derivatives (MANYI-LOH *et al*., 2011a). Less frequently, putative markers of geographic origins have also been detected (RADOVIC *et al*., 2001).

e-Noses have been effectively employed in discriminating honeys from different botanical and/or geographical origins (BENEDETTI *et al*., 2004; DYMERSKI *et al*., 2014; ZAKARIA *et al*., 2011). Sometimes the e-Nose was coupled to another technique, such as Fourier Transform-Infra Red (FT-IR) spectroscopy (SUBARI *et al*., 2012) or Electronic Tongue (BURATTI *et al*., 2004). The coupling of analytical techniques based on different physical-chemical principles and the "merged" dataset thus generated often allowed for an enhancement in discrimination capability.

Recently, PTR-MS coupled to a quadrupole mass analyzer was employed in the classification of honeys having different botanical origins (KUŚ and VAN RUTH, 2015): the technique was not always able to perform a correct classification, affording an average prediction accuracy of 77%. In another recent paper (SCHUHFRIED *et al*., 2016), a PTR-MS

instrument using a Time-of-Flight (ToF) mass analyzer was employed in the headspace analysis of 70 mono-floral honeys of diverse origins. The higher mass resolution of the ToF detector, along with the use of multivariate classification techniques, provided 90-100% correct predictions based on botanical origin.
The assessment of food typicality represents an issue of major relevance to the food industry and a challenging task from an analytical point of view. The objective of the present work is thus twofold: (i) find new analytical tools for the valorization of local food production and (ii) compare the performance of two e-Noses based on different physical-chemical principles. Quite interestingly, the two instruments provide similar performance, with advantages and drawbacks on both sides: the MOS-based e-Nose is more portable and low-cost but it does not provide information as to sample headspace composition, whereas the MS-based instrument gives a fairly detailed analytical insight.

2. MATERIALS AND METHODS

2.1 Honey samples

Honey commercial samples were provided by the Servizio Veterinario dell'Azienda Sanitaria dell'Alto Adige (Bolzano, Italy). The sample set consisted of 26 honeys, out of which 14 originating from South Tyrol and 12 from other European countries (namely Italy, Romania, Spain, Germany and Czech Republic). From the point of view of botanical origins, the sample set was rather heterogeneous, including multi-flower and forest samples as well as monofloral honeys (namely from acacia, chestnut, dandelion, lime and eucalyptus). The year of production of the honeys was 2013. All samplings were performed from the same jar, typically containing 250-500 g of sample. For a more detailed description of the sample set please refer to Table 1, supplementary material.

2.2 PTR-ToF-MS

Headspace measurements were performed using a commercial PTR-ToF 8000 instrument (Ionicon Analytik GmbH, Innsbruck, Austria). The instrumental conditions in the drift tube were the following: drift voltage 550 V, drift temperature 110°C, drift pressure 2.33 mbar affording an E/N value of 140 Townsend (1 Td = 10^{-17} V*cm^2). Sampling was performed with a flow rate of 40 sccm using a heated (110°C) PEEK transfer line. Measurements were performed in an automated fashion by means of a multipurpose GC automatic sampler (Gerstel GmbH, Mulheim am Ruhr, Germany). The analytical method was mutuated from a previously validated method for coffee powder headspace analysis (YENER *et al*., 2014), with some minor adaptations (not shown). Honey aliquots (1.0 g) were transferred into 40-ml glass screw-capped vials, suitable for volatile analysis. All measurements were performed in triplicate. The measurement order was randomized to avoid possible systematic memory effects. All vials were incubated at 40°C for 30 min before PTR-MS analysis. Each sample was measured for 30 s, at an acquisition rate of one mass spectrum per second. Data processing of ToF spectra included dead time correction, internal calibration and peak extraction steps performed according to a procedure described elsewhere (CAPPELLIN *et al*., 2010). In this case this allowed to reach a mass accuracy better than 0.001 Th, which is sufficient for sum formula determination. The baseline of the mass spectra was removed after averaging the whole measurement and peak detection and peak area extraction was performed by using modified Gaussian to fit the data (CAPPELLIN *et al*., 2011). To determine the concentrations of volatile compounds in ppbv = nL of VOC L^{-1} headspace the formulas described by LINDINGER and JORDAN

(1998) were used by assuming a constant reaction rate coefficient (k_R=2×10^{-9} cm^3/s) for H_3O^+ as a primary ion.
The PTR-ToF-MS dataset was submitted to an initial step of filtration based upon concentration. The selection of mass peaks exceeding an arbitrary threshold has often proven to be an effective empirical approach to improve the discrimination ability of PTR-MS data (APREA *et al*., 2015). A concentration threshold arbitrarily set at 1 ppbV, was applied. When peaks having an estimated concentration higher than 1 ppbV were selected, a subset of 55 peaks was generated. For the purpose of multivariate analysis, the mass spectral fingerprint obtained for every sample was normalized by the corresponding total emission. Normalization has already been proven useful (YENER *et al*., 2015), generally allowing to compensate variations in total emission, at the same time preserving the mass spectral fingerprint typical of each sample or sample class.

2.3 Electronic Nose

Analyses were performed with a PEN3 e-Nose (Airsense Analytics, Schwerin, Germany). The instrument has ten Metal Oxide Semiconductor (MOS) sensors displaying different specificity profiles (Table 2). The e-Nose was equipped with an automated sampling device (headspace sampler HSS32 from Airsense Analytics). The analytical procedure for e-Nose honey analysis was validated in a previous work (ZULUAGA *et al*., 2011). Honey samples were measured directly, with no prior dilution. One-gram aliquots were transferred into 10-ml vials, suited for volatile compound analysis. Samples were equilibrated for 20 minutes at 40°C and then analyzed. The e-Nose program was based upon measurement cycles of 150 seconds, separated by 450 seconds of sensor flushing with clean air. Inlet flow was set at 400 ml/min. E-Nose sensor specificities, as stated by the producer, are reported in Table 2.

Table 1: Main characteristics of the honey samples used in the study.

Sample designation	Geographic origin	Botanical origin
H01	South Tyrol	acacia
H02	South Tyrol	forest
H03	South Tyrol	forest
H04	South Tyrol	multiflower
H05	South Tyrol	forest
H06	South Tyrol	mixed (forest/flower)
H07	South Tyrol	multiflower
H08	South Tyrol	multiflower
H09	South Tyrol	multiflower
H10	South Tyrol	chestnut
H11	South Tyrol	multiflower
H12	South Tyrol	multiflower
H13	South Tyrol	multiflower

H14	South Tyrol	multiflower
H15	South Tyrol	multiflower
H16	South Tyrol	dandelion
H17	South Tyrol	multiflower
H18	South Tyrol	multiflower
H19	Italy	chestnut
H20	Italy	chestnut
H21	Italy	lime
H22	Italy, Romania	acacia
H23	Italy, Spain, Romania	forest
H24	Italy	eucalyptus
H25	EU	multiflower
H26	Germany	multiflower
H27	Czech Republic	multiflower
H28	Czech Republic	multiflower
H29	Czech Republic	forest

Table 2: E-nose sensor specificities, as declared by the manufacturer.

Sensor	**Specificity**
S1	Aromatic compounds
S2	Broad range, very sensitive, reacts with nitrogen oxides
S3	Ammonia, aromatic compounds
S4	Mainly hydrogen, selectively (breath gases)
S5	Alkenes and less polar aromatic compounds
S6	Methane, broad range
S7	Sulfur compounds, terpenes, limonene and pyridine
S8	Alcohols, broad range
S9	Sulfur organic compounds
S10	Reacts on high concentrations (>100ppm), sometimes very selective (methane)

2.4. Software

All data analysis was performed using MATLAB (Statsoft, Natick, MA) and R software (the R Foundation for Statistical Computing, Vienna, Austria).

2.5. Statistical analysis

All results are to be intended as means of triplicate measurements. For Principal Component Analysis (PCA), all variables were normalized using the respective standard deviations. Linear Discriminant Analysis (LDA) was carried out with the aid of the R package MASS (Venables, Ripley, and Venables 2002). LDA models were cross-validated by "leave-one-out" method.

3. RESULTS AND DISCUSSIONS

3.1. Classification of honey samples based on Metal Oxide Sensors

In this study, the headspace of 26 honey samples was analyzed with a portable electronic nose. Such device employed an array of ten electronic gas sensors (metal oxide sensors) able to detect and distinguish headspace volatiles via a pattern-recognition algorithm. Typical signals generated by the e-nose with honey samples are shown in Fig. 1.

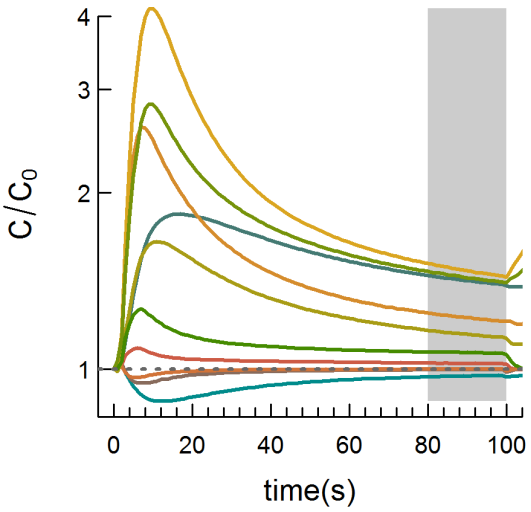

Figure 1: Typical e-Nose profile obtained on a honey sample. Signals were averaged between 80 and 100 seconds, as indicated by the shaded area.

The responses from each sensor during 120 s allowed to extract two data: the maximum signal and a plateau value (recorded arbitrarily between 80 and 100 s of measurement). Based on a preliminary data analysis (results not shown), the two data sets displayed a high degree of covariance; the plateau values, which displayed better repeatability, where thus used in subsequent analysis, whereas maximum values were discarded. Fig. 2 shows the score plot of the principal component analysis (PCA). PCA is an unsupervised pattern recognition tool very useful to plot in a reduced dimensional space (*i.e.* generally, the first two principal components are sufficient to explain most of the variance contained in the original dataset), observe any potential similarities between the samples and, in case, identify the most important variables responsible for such similarities. In detail, the score values of the first two principal components (accounting for the 91% of the total

variability) allow to establish a relatively good separation between samples from South Tyrol from those of other origins, mostly obtained through the employment of component 2.
Looking at the loading values (Fig. 2), the observed discrimination capacity is mainly explained by just 5 metal oxide sensors, and namely S2, S6, S7, S8 and S9. The higher response obtained with sensor S8 in honeys of various European origins is in agreement with mass spectrometric data, showing a higher ethanol content (paragraph 3.2). Sensors S7 and S9, which are specific for sulfur compounds, show more intense signals in South Tyrol honeys. Instead, sulfur compounds do not seem to be important for honey discrimination by PTR-MS, thus suggesting that the analytical responses provided by the two e-Noses are somewhat complementary.

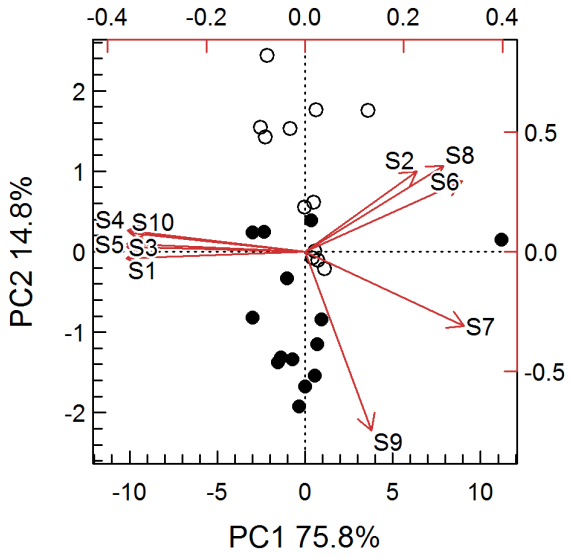

Figure 2: Principal Component Analysis of the autoscaled data obtained by e-Nose (● = South Tyrol, ○ = Other). Alphanumeric codes correspond to e-Nose sensors.

3.2. Classification of honey samples based on PTR-ToF-MS

We next investigated the potential enhancement offered by a more advanced electronic nose based on mass spectrometry. Here we used an on-line proton-transfer-reaction mass spectrometry based on hydronium ions as ion source reagents directly connected to an analyzing time-of-flight mass spectrometer system (PTR-ToF-MS). Fig. 3 shows a typical mass spectrum obtained for a honey sample, in the range 15-215 Th of mass-to-charge ratio (*m*/*z*). The high mass resolution provided by the Time-of-Flight mass analyzer enabled the detection of more than 204 mass peaks**.** Upon filtering based on average concentration a subset of 55 mass peaks, having concentrations higher than 1ppbV, was selected and employed in further analyses.

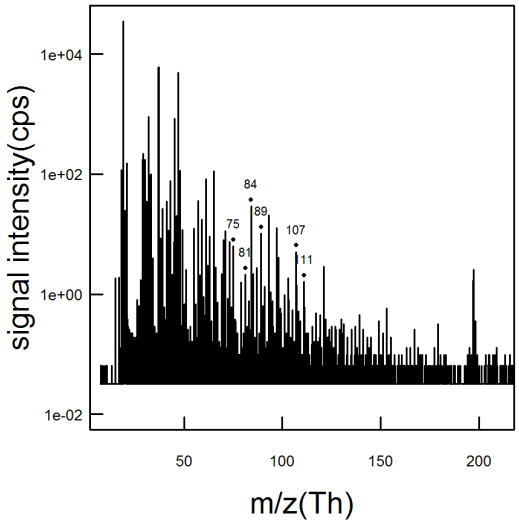

Figure 3: Typical mass spectrum obtained on a honey sample. The position of some selected mass peaks is highlighted by the corresponding nominal masses.

PCA was next used to reduce the dataset dimensionality, observe sample similarities and highlight the most important mass fragments (Fig. 4). The score values of the first two principal components (accounting for the 53% of the total variability) allow visualize a partial separation between the samples from South Tyrol respect those from other origin. Separation was achieved thanks to both Principal Components 1 and 2. The visual inspection of Principal Component higher than two did not provide an improvement in discrimination ability. To understand which mass fragment is responsible for the observed clustering of the samples, the loading values were then analyzed; a subset of 10 mass peaks was defined (Fig. 4), referring to the variables whose loadings showed the highest absolute values for either Principal Component.

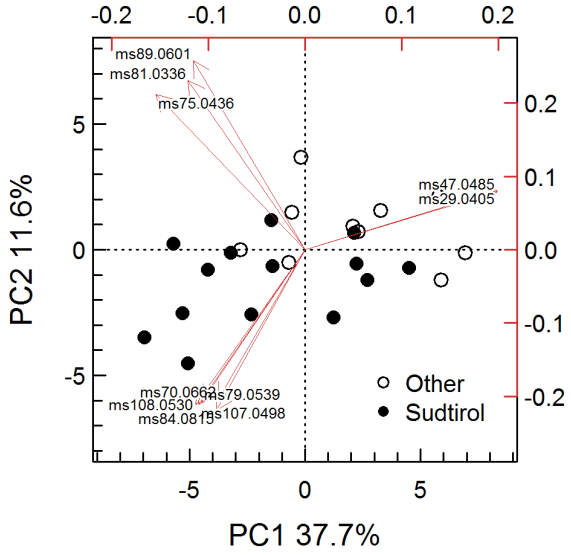

Figure 4: Principal Component Analysis of the autoscaled data obtained by PTR-ToF-MS (● = South Tyrol, ○ = Other). Alphanumeric codes correspond to mass-to-charge ratios as measured by PTR-ToF-MS.

The mass spectrometric data obtained by means of the PTR-TOF has a resolution of 4000 Dm/m or higher. This, after calibration, typically allows for the determination of masses up to the third decimal digit, eventually ensuring the assignment of a sum formula to most mass peaks. The cross-matching of mass spectral data with published databases of honey volatiles (KAŠKONIENĖ and VENSKUTONIS 2010; MANYI-LOH, Ndip, and Clarke 2011b; WOLSKI *et al*. 2006) and fragmentation patterns of pure compounds (APREA *et al*. 2007; BUHR, van Ruth, and Delahunty 2002; DEMARCKE *et al*. 2009) allowed to tentatively assign some of the detected mass peaks to known constituents of the headspace of honey. All these compounds are well representative of factors having a key impact in affecting honey quality and characteristics such as floral origin, oxidation, fermentation *etc*. Mass peaks *m*/*z* 84.082 (with a fragment at *m*/*z* 70.066), *m*/*z* 81.034 and *m*/*z* 111.044 were tentatively attributed to an N-heterocycles and carbonyls which, even though not necessarily reported in honey, are known as Maillard reaction intermediates and might participate in non-enzymatic browning reactions that take part in the oxidative alteration of many food products, including honey (NURSTEN 2005). Mass peak *m*/*z* 107.049 (along with fragment at *m*/*z* 79.054 and 13C isotopologue at *m*/*z* 108.053) was tentatively assigned to benzaldehyde, a compound previously reported in some unifloral honeys (MOREIRA and DE MARIA 2005) and associated to almond and burnt sugar sensory notes (ACREE and ARN 2004). Other relevant mass peaks could be assigned to well-known fermentation products already detected in honey (WOLSKI *et al*. 2006). They namely were ethanol (*m*/*z* 47.048 and fragment at *m*/*z* 29.040), methyl-acetate and methyl-formate (*m*/*z* 75.044), and acetoin, butyric acid, butyrolactone and ethyl-acetate (*m*/*z* 89.060 and fragment *m*/*z* 71.049).

3.3. Comparison of the e-Noses

Two classification models based on linear discriminant analysis (LDA) were next build up on the basis of the most important variables selected by PCA for e-Nose based on MOS sensors and PTR-TOF-MS, respectively. LDA is a supervised pattern recognition tool especially developed for qualitative classification problems. When the signal from the selected MOS sensors were used, the resulting model afforded correct identifications for 82% and 87% of South Tyrol honeys and samples of other origin, respectively, with an overall 85% of correct identifications. Instead, when the model was built with the selected fragments from PTR-TOS-MS, then, the resulting classification model was able to correctly identify 92% and 78% of honeys samples from South Tyrol and other origin, respectively, with an overall 85% of correct identifications. Further detail about the discrimination is provided in the supplementary material (Table 3). The results show that a portable e-Nose, once validated, may have classification performance similar or even better than that achievable with instruments based on high resolution mass spectrometry. Contrarily to what expected, the higher amount of fragments detected by the high resolution mass spectrometers do not result with a higher capacity to discriminate samples. Apparently, the capacity of discriminating the samples is hindered by an increased noise or uncertainty around the signal of individual fragments.

Table 3: LDA confusion matrices, as obtained using e-Nose and PTR-ToF-MS (left and right, respectively).

		Model based upon MOS-based e-Nose		**Model based upon PTR-ToF-MS**	
		Predicted		**Predicted**	
Original		**Other**	**South-Tyrol**	**Other**	**South-Tyrol**
	Other	10	2	11	1
	South-Tyrol	2	12	3	11

4. CONCLUSIONS

This research work presents an unprecedented analytical approach based on two different types of electronic nose. This approach was employed to address the question of the typicality of honeys from South Tyrol (Italy). PTR-ToF-MS, with its high mass resolution, allowed for a rapid, yet thorough characterization of the honey headspace, permitting to pinpoint several candidate aromatic markers. The MOS-based electronic nose provided a cost effective solution to the same problem, being more portable and less expensive than the latter instrument. The sample set was of limited size (26 honeys) and the work is thus intended to be a preliminary study. Botanical diversity, which is known to play a major role on headspace composition, was probably underestimated, and the corroboration of these first results by means of a more extended survey is indeed advisable.
The work also addresses the strategic theme of the typicality of food products issued from a small alpine region (South Tyrol). The work demonstrates how the development of novel analytical approaches can enable researchers and institutions to validate the typicality of regional products, representing an undoubted source of added value to all local productions.

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