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Validation of honey-bee smelling profile by using a commercial electronic nose

Validación de la técnica de nariz electrónica para la determinación del perfil olfativo de miel de abejas

Ana R. Correa¹, Martha M. Cuenca², Carlos M. Zuluaga³,
Matteo M. Scampicchio⁴, and Marta C. Quicazán⁵

ABSTRACT

Honey is a natural sweetener and its quality labels are associated to its botanical or geographical origin, which is being established by palynological and sensorial analysis. The use of fast and non-invasive techniques such as an electronic nose can become an alternative for honey classification. In this study, the operational parameters of a commercial electronic nose were validated to determine the honey odor profile. A central composite design with five factors, three levels and 28 assays was used, varying sample amounts (1, 2 and 3 g), incubation temperature (30, 40 and 50 °C), incubation time (30 min), gas flow (50, 150 and 250 mL/min) and injection time (100, 200 and 300 s). The commercial nose had ten sensors. Repeatability was evaluated with a coefficient of variation of 10%. The response surface methodology was used and the optimal operating conditions were: 3 g of sample, incubation at 50 °C for 17 min, gas flow of 100 mL/min and sampling time of 150 s. Finally, these parameters were used to analyze 19 samples of honey, which were classified according to their odor profiles, showing that it can be a useful tool to classify honey.

Keywords: Electronic nose, honey-bee, validation and smelling.

RESUMEN

La miel es utilizada como edulcorante natural. El origen botánico o geográfico de las mieles se establece mediante análisis palinológico y sensorial. El uso de técnicas rápidas como la nariz electrónica puede ser una alternativa para la clasificación de mieles. En este estudio se validaron los parámetros operativos de una nariz electrónica comercial para determinar el perfil del olor de miel. Se utilizó un diseño compuesto central con cinco factores, tres niveles y 28 ensayos, variando la cantidad de muestra (1, 2 y 3 g), la temperatura de incubación (30, 40 y 50 °C), el tiempo de incubación (10, 20 y 30 min), el flujo de gas (50, 150 y 250 mL/min) y el tiempo de inyección (100, 200 y 300 s). La nariz comercial contaba con diez sensores. La repetibilidad se evaluó con un coeficiente de variación de 10%. Se utilizó la metodología de superficie de respuesta y se encontraron las siguientes condiciones: 3 g de muestra, incubación a 50 °C por 17 min, flujo de gas de 100 mL/min y tiempo de muestreo de 150 s. Finalmente, estos parámetros se utilizaron para analizar 19 muestras de miel, las cuales se clasificaron según sus perfiles de olor, demostrando así que puede ser una herramienta útil para clasificar mieles.

Palabras clave: Miel, nariz electrónica, validación y perfil olfativo.

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Introduction

Food volatile compounds analysis is very important and, normally, it is related to smell, which is one of the most important sensory parameters. Generally, volatile compounds analysis is performed by using gas-chromatographic methods, which are robust and powerful (Agila & Barringer, 2012; Castro-Vázquez, Díaz-Maroto, González-Viñas, & Pérez-Coello, 2009;

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Papotti, Bertelli, & Plessi, 2012), but it is necessary to preprocess sample which is time-consuming and it is difficult to determine it.

Electronic Noses (e-Noses) are an alternative, and generally they have an electrochemical sensors array that provides a fingerprint of a given sample headspace (Romano *et al.*, 2016). 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 (Gliszczynska-Swiglo & Chmielewski, 2016). The e-Nose provides little information as to the actual composition of the sample headspace but they are generally easy to use, they provide a high analytical throughput and they are relatively inexpensive.

Honey is a traditional natural product produced by bees from the nectar of flowers (Gómez-Díaz, Navaza, & Quintáns-Riveiro, 2012). Its physicochemical, microbiological and sensory characteristics are associated with bee species, botanical and geographical nectar origin, harvesting practices, extraction and honey storage (Castro-Vázquez, Díaz-Maroto, de Torres, & Pérez-Coello, 2010).

Honey contains mainly sugar and water, but also a great variety of volatile compounds and there have been reported like 400 different compounds for a single type of honey (Piana *et al.*, 2006; Romano *et al.*, 2016). There are different methods for determining physical, chemical, microbiological quality, and botanical origin (Bogdanov *et al.*, 2004; Castro-Vázquez *et al.*, 2007; Cuevas-Glory *et al.*, 2007; Kuš & van Ruth, 2015; Montenegro *et al.*, 2008; Piana *et al.*, 2006), which can be related to honey sensory attributes such as color, texture, flavor and appearance. E-Noses applied to honey characterization and classification represent the application of a novel, rapid and non-invasive technique, becoming a useful tool for quality control, shelf-life and food adulteration (Ampuero, Bogdanov, & Bosset, 2004; Arvanitoyannis, Chalhoub, Gotsiou, Lydakis-Simantiris, & P., 2012; Benedetti, Mannino, Sabatini, & Marcazzan, 2004; Čačić, Primorac, Kenjerić, Benedetti, & Mandić, 2009; Gliszczynska-Swiglo & Chmielewski, 2016; Quicazán, Zuluaga, & Díaz, 2014; Romano *et al.*, 2016; Subari, Saleh, Shakaff, & Zakaria, 2012; Zuluaga *et al.*, 2015).

In order to determining a smelling profile with an electronic nose, its operating conditions have to be considered: sample temperature (depending on amount and volatility of compounds presents), sampling time, gas flow, incubation time, and cleaning time of the sensors (Quicazán *et al.*, 2014). This study aimed to validate the operating conditions for a commercial electronic nose PEN 3 (Airsense, Germany) to obtain smelling profiles for honey-bee samples, demonstrating that it can be a portable and low-cost technique even if it does not provide quantitative information about sample headspace composition.

Methodology

Honey samples

For validation, it was used an Acacia honey (*Robinia pseudoacacia*) from the local market of Bolzano (Italy). For performing the final test with different types of honey, there were used 19 different honeys from different places, presented in Table 3.

Determination of volatile compounds profile for electronic nose

It was used a portable commercial electronic nose Airsense PEN 3 (Airsense, Germany), with an array of 10 semiconductor sensors (Table 1). Honey samples were served and weighed into glass vials of 10 mL. The vials were hermetically sealed with lids containing septa silicone. Operating parameters were changed manually for each test. The obtained responses were recorded by the sensors through Win Muster software (Airsense, Germany), and quantitatively expressed as a conductance value. It was obtained a data matrix of “m” columns “n” rows, where “m” columns represent the number of sensors of the electronic nose and “n” the number of times the analysis was performed. From the matrix for each sensor, it was obtained the medium coefficient differential value nuance response curve of each sensor corresponding to the value of the differential coefficient (mcdv) calculated by using Equation (1) (Yin & Tian, 2007).

$$mcdv = \frac{1}{N-1} \sum_{i=1}^{N-1} \frac{x_{i+1} - x_i}{\Delta t} \quad (1)$$

Where mcdv is the result of the characteristic value for each sensor profile of each sample, N is the number of time intervals analyzed, x_i and x_{i+1} result of conductance in times i and $i+1$, respectively; Δt is the time interval between conductance data, which by default is 1s. The values obtained reflect the average speed of sensors responses and represent their principal characteristics (Quicazán *et al.*, 2014).

Table 1. Symbols and groups of compounds detected by each E-nose sensor

Sensor	Symbol	General description
1	W1C	Aromatic compounds
2	W5S	Nitrous oxide and ozone
3	W3C	Aromatic compounds
4	W6S	H ₂ , O ₂ y CO ₂
5	W5C	Alkanes
6	W1S	Methane
7	W1W	Terpens and organosulfur compounds
8	W2S	Alcohols
9	W2W	Organosulfur compounds
10	W3S	Methane and aliphatic compounds

Source: Authors

Operating parameters evaluation

A central composite design with five factors with three levels (Table 3) and 28 trials were used. Responses were conductance values for each sensor (10) of the E-nose. Response surface methodology was used and optimal operating conditions were found by a responses optimization design.

Table 2. Factors and levels of the central composite design

Factors Levels	Amount of sample (g)	Incubation temperature (°C)	Incubation time (min)	Gas flow (mL/min)	Injection time (s)
-1	1	30	10	50	100
0	2	40	20	150	200
+1	3	50	30	250	300

Source: Authors

Repeatability evaluation

Smelling profile of 10 samples of acacia honey (*Robinia pseudoacacia* L.) from the same batch were determined by using optimal operating conditions. It was used 10% maximal variation coefficient (VC) criteria to evaluate

repeatability, which measures a dispersion that correlates the average ($\bar{X}$) and the standard deviation (s) according to Equation (2):

$$VC = \frac{S}{\bar{X}} \times 100\% \quad (2)$$

Honey classification with optimized parameters

Smelling profile of 19 different honey samples were performed by using optimized e-nose parameters. 10 replicates were performed. With average mcdv values, a Principal Components Analysis was performed.

Results and discussion

Smelling profiles

The mcdv for all sensors in each of 28 trials (Table 4) were calculated from the data matrix obtained by using Equation (1). All sensors recorded conductance values different for each of the tests performed, demonstrating all conditions reflect different responses.

Table 3. Different honey samples used for classification

Sample Number	Common Name	Botanical Origin	Geographical origin	Bee Species	Production year
1	Chestnut	<i>Castanea sativa</i>	Como - Italy	<i>Apis mellifera</i>	2014
2	Acacia	<i>Robinia pseudoacacia</i>	South Tyrol - Italy	<i>Apis mellifera</i>	2015
3	Honey Mixture Ambrosolio	Imported honey mixture	Hungary, Italy, Ukraine	<i>Apis mellifera</i>	-
4	Rhododendron	<i>Rhododendron ferrugineum</i>	Sondrio - Italy	<i>Apis mellifera</i>	2015
5	Saxifraga	<i>Saxifraga corsica</i>	Como - Italy	<i>Apis mellifera</i>	2015
6	Berseem	<i>Trifolium alexandrinum</i>	Como - Italy	<i>Apis mellifera</i>	2015
7	Eucalyptus	<i>Eucalyptus globulus</i> Labill	Sardinia - Italy	<i>Apis mellifera</i>	2015
8	Zulla	<i>Hedysarum Coronarium</i>	Chieti - Italy	<i>Apis mellifera</i>	2015
9	Thyme	<i>Thymus vulgaris</i>	Sicily - Italy	<i>Apis mellifera</i>	2014
10	Apenine Honeydew	Forest Honeydew	Bolognese appennine	<i>Apis mellifera</i>	2014
11	Hill Honeydew	Forest Honeydew	Bolognese appennine	<i>Apis mellifera</i>	2014
12	Orange blossom	<i>Citrus aurantiifolia</i>	Coquena - Italy	<i>Apis mellifera</i>	2015
13	Heather	<i>Erica arborea</i>	Corse - Italy	<i>Apis mellifera</i>	2015
14	Sunflower	<i>Helianthus annuus</i>	Ancona - Italy	<i>Apis mellifera</i>	2015
15	Lime	<i>Tilia europaea</i>	Como - Italy	<i>Apis mellifera</i>	2015
16	Mixed flower Italian	Mixed flower Italian	Como - Italy	<i>Apis mellifera</i>	2015
17	Ailanthus	<i>Ailanthus altissima</i>	Chieti - Italy	<i>Apis mellifera</i>	2015
18	Mixed flower Colombian	Mixed flower Colombian	Sierra Nevada de Santa Marta - Colombia	<i>Apis mellifera</i>	2015
19	Colombian Tetragonisca angustula	Mixed flower Colombian	Medellín - Colombia	<i>Tetragonisca angustula</i>	2015

Source: Authors

Table 4. MCDV for each sensor response at each trial

Trial numbers	Semiconductor sensors									
	W1C	W5S	W3C	W6S	W5C	W1S	W1W	W2S	W2W	W3S
1	1,0290	1,2794	1,0529	0,9253	1,0590	0,9234	1,7277	0,9738	1,5317	1,0060
2	1,0329	1,4880	1,0530	0,8484	1,0585	0,8751	2,0414	0,9453	1,6778	1,0043
3	1,0089	1,2742	1,0229	0,9879	1,0217	1,0315	1,7468	1,0071	1,4792	0,9997
4	0,9906	1,4973	1,0159	0,9853	1,0196	1,1936	1,9991	1,0685	1,6072	1,0017
5	1,0433	1,2801	1,0579	0,9259	1,0604	0,8412	1,7638	0,9393	1,5311	1,0043
6	1,0232	1,4574	1,0368	0,8565	1,0394	0,8898	2,0066	0,9478	1,6080	1,0061
7	1,0028	1,3112	1,0190	0,9912	1,0198	1,0904	1,7980	1,0265	1,4894	1,0004
8	1,0047	1,4410	1,0233	0,9726	1,0240	1,0891	2,1203	1,0474	1,6663	0,9988
9	1,0150	1,7866	1,0447	0,9494	1,0550	1,0484	2,6877	1,0505	2,0278	1,0652
10	1,0187	2,1960	1,0475	0,8714	1,0575	0,9804	3,0588	1,0281	2,1803	1,0633
11	0,8209	2,9271	0,9420	1,0086	1,0084	2,5684	2,8930	1,6541	2,3492	1,0413
12	0,8259	2,9712	0,9533	1,0058	1,0142	2,5060	2,9714	1,6421	2,2753	1,0411
13	0,8654	2,7288	0,9921	0,9287	1,0460	2,0361	3,7534	1,5244	2,7741	1,0158
14	0,8635	2,9143	1,0002	0,8614	1,0516	2,1111	3,9044	1,5293	2,7607	1,0112
15	0,9027	2,3699	0,9840	0,9925	1,0127	1,9573	3,3322	1,3849	2,4085	1,0096
16	0,9098	2,4230	0,9914	0,9878	1,0168	1,8885	3,5582	1,3849	2,4670	1,0057
17	0,9636	1,8021	1,0282	0,9940	1,0421	1,4451	2,4685	1,1831	1,8951	0,9945
18	0,9367	2,4101	1,0181	0,9653	1,0394	1,7029	3,2711	1,2940	2,3300	1,0101
19	0,8719	2,6609	0,9890	0,9683	1,0330	2,1231	3,2022	1,5039	2,3480	1,0048
20	0,9316	2,1596	1,0149	0,9691	1,0382	1,5497	2,8664	1,2365	2,1230	1,0092
21	0,8239	3,0150	0,9707	0,8932	1,0400	2,1871	4,5651	1,6934	3,2981	1,0175
22	0,8142	2,8177	0,9318	0,9725	0,9853	2,4987	3,7252	1,6803	2,6415	0,9959
23	0,8391	2,8049	0,9678	0,9842	1,0292	2,5308	3,8153	1,7181	2,8268	1,0096
24	0,9521	2,0683	1,0254	0,9187	1,0432	1,4197	2,8284	1,1901	2,0654	1,0124
25	0,9691	1,7907	1,0278	0,9801	1,0406	1,4851	2,5044	1,2227	1,9271	1,0096
26	0,8452	2,8959	0,9773	0,9824	1,0327	2,5715	3,7819	1,7128	2,7503	1,0145
27	0,8467	2,8304	0,9763	0,9643	1,0306	2,4656	3,8415	1,6835	2,8070	1,0096
28	0,8851	2,4618	0,9958	0,9683	1,0351	2,1887	3,4161	1,5358	2,5174	1,0051

Source: Authors

Operating parameters evaluation

The highest statistically significant changes in relation to each factor and interactions between factors were evaluated. With a responses optimization design, it was found that the best operating conditions were 3 g sample, incubation temperature 50 °C, incubation time 1020s, gas flow of 100mL/min and 150s sampling time, result that confirming the importance not only of the sample but also of operating conditions.

Table 5 presents the statistical results of p-value from the response surface for each of factor and interactions among factors. Values $p < 0,05$, indicate the factor or

interaction between two factors has a greater influence on the response of each sensor. Therefore, it was found that incubation, temperature, gas flow, injection time, interaction between incubation time and injection time, interaction between amount of sample and injection time have greater influence on the responses. Sensors that react to the presence of methane and aliphatic compounds (W3S), alkanes (W5C) and aromatic compounds (W3C) show the greatest statistically significant changes for each factor and their interactions, which is correlated to the results reported by Cuevas-Glory *et al.* (2007), informing the presence of benzaldehyde, linalool, nonanal and hotrienol.

Table 5. p values for Surface Response Analysis

Factors and interactions	Semiconductor sensors									
	W1C	W5S	W3C	W6S	W5C	W1S	W1W	W2S	W2W	W3S
Incubation temperature (°C)	0,000	0,000	0,000	0,009	0,004	0,000	0,000	0,000	0,000	0,000
Incubation time (min)	0,693	0,703	0,958	0,152	0,042	0,986	0,005	0,869	0,061	0,000
Injection time (s)	0,001	0,946	0,000	0,000	0,000	0,001	0,010	0,020	0,011	0,000
Amount of sample (g)	0,363	0,704	0,460	0,000	0,089	0,327	0,250	0,240	0,557	0,039
Gas flow (mL/min)	0,063	0,026	0,018	0,754	0,003	0,136	0,049	0,123	0,066	0,071
Incubation temperature (°C) – Incubation time (min)	0,076	0,265	0,667	0,082	0,376	0,146	0,001	0,111	0,005	0,000
Incubation temperature (°C) – Injection time (s)	0,048	0,206	0,021	0,358	0,108	0,040	0,266	0,170	0,746	0,000
Incubation temperature (°C) – Amount of sample (g)	0,766	0,769	0,364	0,848	0,027	0,991	0,906	0,989	0,576	0,251
Incubation temperature (°C) – gas flow (mL/min)	0,477	0,777	0,472	0,564	0,110	0,483	0,584	0,697	0,571	0,359
Incubation time (min) – Injection time (s)	0,000	0,008	0,000	0,382	0,010	0,001	0,752	0,003	0,557	0,000
Incubation time (min) – Amount of sample (g)	0,852	0,521	0,740	0,702	0,170	0,852	0,987	0,872	0,987	0,261
Incubation time (min) – gas flow (mL/min)	0,873	0,409	0,726	0,440	0,068	0,765	0,763	0,785	0,682	0,460
Injection time (s) – Amount of sample (g)	0,871	0,501	0,729	0,000	0,041	0,826	0,920	0,604	0,808	0,317
Injection time (s) – gas flow (mL/min)	0,597	0,369	0,343	0,080	0,043	0,895	0,631	0,822	0,680	0,634
Amount of sample (g) – gas flow (mL/min)	0,000	0,001	0,000	0,162	0,592	0,000	0,557	0,001	0,124	0,000

Source: Authors

P values <0,05 indicate that the factor or interaction between these factors led significant changes in conductance responses obtained by that sensor

Repeatability

mcdv was determined for each of 10 measurements of smelling profile by using Equation (1). From the data matrix, statistical parameters like average, range, standard deviation and coefficient of variation were determined by using Equation (2), as it is shown on Table 6. Variation coefficients of the responses were within the maximal limit (10%). Operating conditions allow obtaining repeatable honey smelling profiles, demonstrating that validation

of a smelling profile depends on the sample but also on sampling conditions.

Honey classification

In Figure 1 is presented the biplot corresponding to a PCA analysis which explains the 80,72% of total variance for 19 honey samples. It is noticeable all samples showed a different smelling profile, especially samples 1, 2, 7 and 12, due to its botanical and geographical origin. Samples 3, 16 and 19 are mixed floral honey and present similar smelling characteristics. Even small smelling characteristics make a difference among honey samples, which is observed by performing their e-nose analysis.

Table 6. Statistical parameters for repeatability evaluation

Statistical parameters	W1C	W5S	W3C	W6S	W5C	W1S	W1W	W2S	W2W	W3S
Minimal value	0,9336	2,2430	1,0229	0,9545	1,0497	1,2362	2,6985	1,0109	2,2498	0,9928
Maximal value	1,0354	2,7667	1,0544	0,9625	1,0580	1,3581	3,1866	1,2609	2,4160	1,0010
Average	0,9839	2,5057	1,0404	0,9581	1,0547	1,2888	2,8655	1,1181	2,3131	0,9970
Standard deviation	0,0379	0,1851	0,0113	0,0032	0,0027	0,0627	0,2032	0,0995	0,0655	0,0026
Coefficients of variation (%)	3,85	7,39	1,09	0,33	0,26	4,86	7,09	8,90	2,83	0,26

Source: Authors

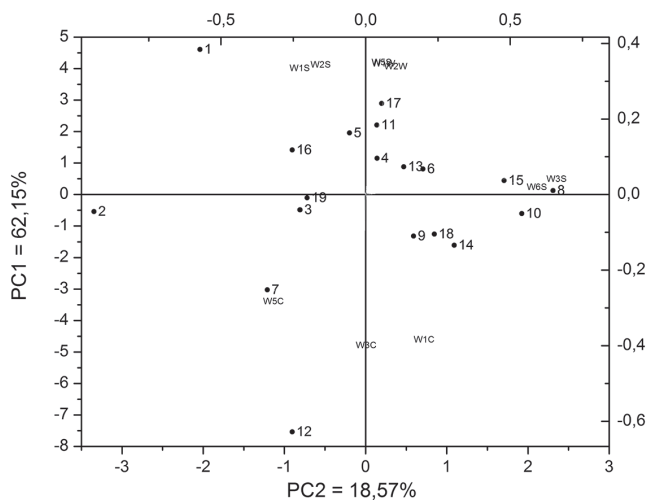


Figure 1. PCA for different kinds of honey using optimized parameters.
Source: Authors

Conclusions

It is concluded that optimized operating conditions found for acacia honey smelling profile were standardized: 3 g sample, incubation temperature 50°C, 1020 s incubation time, gas flow of 100 mL/min and 150 s sampling time, giving repeatable responses for all sensors. Optimized parameters smelling evaluation for 19 different honey samples shows all e-nose sensors give information related to its smelling profile, which is different for all samples, confirming that a validated methodology allows to use this technique as a quick and easy alternative for honey differentiation and classification according to its botanical and geographical origin.

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