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An electronic tongue for honey classification

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Abstract. An electronic tongue system was developed based on 20 all-solid-state potentiometric sensors and chemometric data processing, with polymeric membranes applied on solid conducting silver-epoxy supports and a Ag/AgCl reference electrode. The sensor array was applied to 52 commercial honey samples obtained randomly from different regions of Portugal. These samples were analysed independently for their pollen profiles by biological techniques and the data collected with the tongue were evaluated for discrimination of the samples with multivariate statistical methods (principal component analysis and linear discriminant analysis), to investigate whether the device may provide an analytical alternative for classification of honey samples with respect to pollen type, a task which is time consuming and requires skilled labour when performed by biological techniques. It was found that the tongue has a reasonable efficiency for classification of honey samples of the most common three types (with *Erica*, *Echium* and *Lavandula* as predominant pollens). With linear discriminant analysis, the honey samples yielded about 84% classification accuracy and 72% for crossed validation. In this study, the honey samples correctly classified for the different types of the dominant pollen were: 53% for *Lavandula*, 83% for *Erica* and 78% for *Echium* pollen.

Keywords: Honey; pollen; electronic tongue; multivariate analysis

Multi-sensor arrays that provide global information on complex samples have deserved much interest recently. Instead of measuring specific parameters, these devices acquire global information which, after treatment by appropriate chemometric methods, can be used for multicomponent classification analysis, taste evaluation, etc. Electrochemical sensor arrays or electronic tongues built with non-specific, low-selectivity, chemical sensors with high stability and cross sensitivity to different species in solution, are suitable for analysing complex liquid samples [1]. Electronic tongues or taste sensors based on different electrochemical principles, such as potentiometry [2–6] or voltammetry [7, 8], have been described. Several array types have been tested for potentiometric devices, namely chalcogenide glass sensors [3–5], lipid/polymeric membranes [2, 6] and ion selective membranes [9]. The signal profiles generated by such devices vary with the characteristics of different samples and upon data treatment with multivariate statistical methods for pattern recognition (identification, classification

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and discrimination), allowing qualitative and/or quantitative multi-components analysis, as well as taste evaluation of liquid samples [1].

In this work, an array with 20 potentiometric sensors, based on all-solid-state potentiometric electrodes with polymeric membranes formed on solid conducting silver supports, was constructed and evaluated for discrimination of different types of honeys with different pollen profiles. The chemometric tools used were principal component analysis, an unsupervised pattern recognition technique oftenly used for electronic tongue data treatment [2–4, 6–9], and linear discriminant analysis, a supervised pattern recognition technique, used for sample classification in food analysis, including monofloral honeys characterization [10, 11].

The type of flora where bees collect the nectar together with other factors such as climatic conditions, type of soils, etc., affect the physico-chemical and sensory properties of honey and thus the commercial classification of honey samples. This variability contributes to the existence of different types of honeys (monofloral or polyfloral honeys) with a large variety of specific sensory characteristics. Monofloral honeys, originated predominantly from a single botanical source, have higher demand from the consumer, which means higher commercial value for the producers and raises the question of their quality control.

The honey sensor signal patterns provided by the device were treated by principal component analysis and linear discriminant analysis for honey differentiation, accordingly to the predominant pollens, which in Portugal are of three types: *Erica*, *Echium* and *Lavandula*. In general, a honey sample can be classified as *Lavandula* monofloral honey if its *Lavandula* pollen content is higher than 15%, and as *Erica* or *Echium* monofloral honey if its respective pollen content is higher than 45% [12, 13]. Large variations in pollen content are the result of large differences in the botanical composition of ecosystems around the apiaries. When a honey fulfils the monofloral pollen criteria for more than one species (for instance, >15% *Lavandula* and >45% *Erica*), the classification is based on the physico-chemical properties and organoleptic analysis [14, 15].

The global objective of this line of work is to develop analytical alternatives for honey classification since pollen identification and quantification in honey samples, using biological techniques, is a time consuming task that requires expert labour.

Materials and methods

Reagents

All reagents and solvents were of analytical grade and used as supplied. All solutions were prepared using deionised water. The polymeric membranes, with lipid sensors similar to those used by Toko [2] in a taste sensor, were prepared with poly(vinylchloride) (PVC) from Fluka, as polymeric matrix, 2-nitrophenyl-octylether (2-NPOE) from Fluka, as plasticizer, and tetrahydrofurane (THF) from Fluka, as solvent for the mixture used for constituting the membranes.

The following chemicals were purchased from Fluka: octadecylamine, oleyl alcohol, oleylamine, trioctylmethylammonium chloride (TOMA), tridodecylmethyl-ammonium chloride (TDMA), octadecanoic acid, 1-octadecanesulfonic acid sodium, 1-octadecanethiol, octylamine, 1-dodecanol, 1-tetradecanol, 1-octadecanol, dioctil-phenylphosphate, bis(2-ethylhexyl)phosphate, dodecylamine and dodecanoic acid. 1-dodecanethiol was acquired from Aldrich. Some of the sensor ionophores were those chosen in [2] but others were selected by similarity, based on the nature of functional groups and long carbon chain structure.

Samples

The honey samples (52 samples) were obtained from different places of Portugal, being a representative sampling of the most productive Portuguese honey regions. Prior to use, samples were kept at room temperature. A pollen spectrum analysis (see next section) was carried out for all the samples.

For the electronic tongue analysis, 8–10 g of honey were dissolved in deionised water to obtain a solution with a final concentration of 20% of honey.

Mellisopalynological analysis

The honey pollen spectrum analysis was performed according to the acetolysis Erdtman method [16]. For each analysis, 10 g of honey were diluted with 30 mL of distilled water and, after acetolitic treatment, observed with an optic microscope (Leica DMLB microscope, with 40 × objective). Reference standards obtained from Portugal honey flora were used for grain pollen identification and the samples were classified by their pollinic type according to their found pollen morphology.

Overall, the 52 honey pollen profiles showed a large variety in pollen composition. The pollens found in the samples were: *Echium* (present in 71.2% of the honey samples), *Lavandula* (67.3%), *Erica* (42.3%), *Cytisus* (28.8%), *Prunus* (26.9%), *Leontondon* (15.4%), *Carduus* (15.4%), *Castanea* (9.6%), *Trifolium* (7.7%), *Eucaliptus* (3.8%), *Citrus* (1.9%) and others. The profile determinations showed that *Erica*, *Echium* and *Lavandula* pollen are the three most common types.

Results from the quantitative pollen analysis showed that the analysed samples had always *Erica*, *Echium* or *Lavandula* as the pollen with the highest content. Two of the 52 samples were disregarded because they did not fit in this situation: they showed floral types with *Leontondon* (98%) and *Cardus* (38%) as predominant pollens, probably meaning that they are produced in a geographically restricted area with predominant flora of their respective types. The *Echium* pollen was the predominant in 23 samples, with contents varying from 32 to 91%, and a secondary or tertiary component in 13 other samples (from 6 to 29%). *Lavandula* was found as the main pollen in 15 samples (from 31 to 72%) and as the second-

ary and tertiary component in 16 and 4 samples, respectively. For *Erica*, the values were 12 (from 38 to 100%), 6 and 2, respectively.

Multi-sensor system analysis

Potentiometric measurements were done with a reference electrode Ag/AgCl with double junction (0.5 M K_2SO_4 as external solution) and a multi-sensor analytical system with 20 sensors coupled to a multiplexer Agilent Data Acquisition/Switch Unit model 34970A. Each of the 20 channels was set for DC voltage measurements (± 1 V) with the high impedance option.

Data acquisition was performed with a PC connected to the multiplexer by a RS-232 output and controlled with the Agilent BenchLink Data Logger software. The electric potential pattern response was analysed using a Excel spreadsheet.

All experiments were carried out with the sample in a double wall glass cell thermostatized at 25 °C with a thermostatic bath Tectron Bio from Selecta. Each honey sample measurement with the sensor array was carried out in 15 min.

Construction of the multi-sensor system

The multi-sensor analytical device, a multichannel electrode, was built on an acrylic body (length × width × thickness: 4.2 × 1.7 × 1.0 cm × cm × cm), with 20 holes (with a diameter of 0.3 cm) filled with conducting silver epoxy resin (EPO-TEK E4110) connected to copper electric wires (see Fig. 1).

Each polymeric membrane was constituted from a solution prepared with a mixture of 5% of the sensing material, 65% of the plasticizer (2-NPOE) and 30% of the membrane polymer (PVC), dissolved in a small volume of THF. Each solution was applied to one of the 20 holes of the acrylic body filled with silver resin. Table 1 shows the sensors used to build the polymeric membranes and their exact proportions of mixtures.

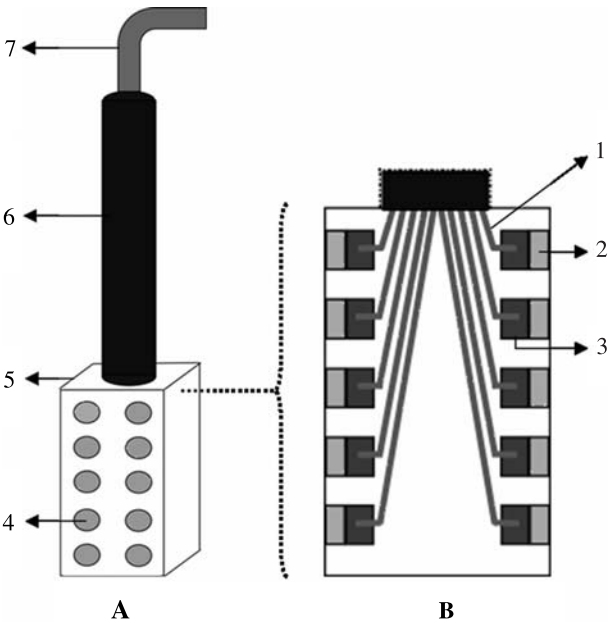

Fig. 1. Scheme of the multi-sensor system developed: (A) front view (back view is similar) and (B) side view; *1* isolated copper wire; *2* polymeric membrane with sensor; *3* conducting silver epoxy resin; *4* hole with polymeric membrane and conducting silver epoxy resin; *5* acrylic body with 20 sensors; *6* plastic tube; *7* cable with 20 isolated copper wires

Table 1. Composition of polymeric membrane mixture

Membrane number	Compound used as sensor	Sensor compound (%)	2-NPOE (%)	PVC (%)
S01	1-octadecanol	5.0	65.0	30.0
S02	oleylalcohol	5.1	64.9	30.0
S03	1-tetradecanol	5.0	65.0	30.0
S04	1-dodecanol	5.0	64.9	30.1
S05	octadecylamine	5.2	64.8	30.0
S06	oleylamine	5.0	65.0	30.0
S07	dodecylamine	5.0	65.0	30.0
S08	octylamine	5.0	64.8	30.2
S09	1-dodecanethiol	5.1	64.5	30.4
S10	1-octadecanethiol	5.2	64.4	30.4
S11	potassium tetrakis(4-chlorophenyl)borate	5.0	64.9	30.1
S12	trioctylmethyl-ammonium chloride (TOMA)	5.0	65.0	30.0
S13	tridodecylmethyl-ammonium chloride (TDMA)	5.0	65.0	30.0
S14	DOP + TOMA	5.0*	64.9	30.1
S15	DOP + TDMA	5.0*	64.9	30.1
S16	dioctylphenil-phosphate (DOP)	5.2	64.8	30.0
S17	undecanoic acid	5.0	65.0	30.0
S18	dodecanoic acid	5.1	64.9	30.0
S19	octadecanoic acid	5.0	65.0	30.0
S20	oleic acid	5.0	65.0	30.0

* Sensor mixture with 1:1 mass proportion.

Statistical analysis

The potentiometric sensor signals were standardized using the interval normalization for the global raw data of each day.

Principal component analysis (PCA) was applied to the signal pattern provided by the device to visualise the differences between the honey samples and allow the grouping or classification of the honey samples. The α-Cronbach's coefficient was used to verify the internal consistency of the components.

Linear discriminant analysis (LDA) was also performed to obtain classification rules for differentiation between honey samples accordingly to the most predominant pollen (*Erica*, *Echium* and *Lavandula*).

Data were tested for normality and homogeneity of variances in order to verify the assumptions of the analysis of variance (ANOVA). As multivariate normal tests are difficult to implement, the Kolmogorov-Smirnov with Lilliefors significance correction and the Shapiro-Wilk tests were used to evaluate the normality of the distribution of the sensors signals for the three groups of honey considered. The homogeneity of variance was tested using the Levene test. One-way ANOVA with or without Welch's test was used to test the significance of the honey group means for each independent variable (each sensor response) that was measured, to verify if there exists at least one group for which the means are different. The Wilks' Lambda test was applied to verify which canonical discriminant functions were significant [17].

All statistical analysis were performed at a significance level of 5% using the SPSS and JMP softwares [17–19].

Results

The response electric potentials measured by the electronic tongue were obtained for the complete set of 52 honey samples.

The device showed a fast and stable response after 5 min of signal acquisition. As the signal of some sensors showed time-oscillation it was decided to use, as the experimental response, the average of the electric potential signals calculated using the values obtained for the last minute of the assay.

Typical electric potential patterns for 6 honey samples with different predominant pollen (*Erica*, *Echium* and *Lavandula*) are presented in Fig. 2. As can be seen from this figure, honey samples with *Lavandula* or *Echium* as predominant pollen presented similar signal profiles, being different from those obtained for samples where *Erica* pollen predominates.

Principal component analysis showed that 95.3% of the total variance of the data could be explained using only three principal components. A fourth dimension was not considered since it gave an eigenvalue lower than 1 and a negative value of the α-Cronbach parameter. The number of principal components to be extracted was confirmed by the Scree plot.

The representation of the first two principal component scores of the electronic tongue signals obtained for the 50 honey samples retained for data analysis is presented in Fig. 3. This figure shows that honey samples could be separated in four groups. Group 1 contains only honeys with *Lavandula* as the most predominant pollen (eight honey samples); group 2 includes sixteen *Echium* honeys and two honey samples with higher content in *Lavandula* pollen but with *Echium* as second pollen; group 3 includes mainly nine honeys of *Erica* type, but also three other samples, two with *Lavandula* and one with *Echium* as principal pollen; finally, group 4 is a mixed group with twelve honeys of the three kinds of pollen. The first and second groups, in the positive part of the first principal component, are separated by the second principal component (also in the positive region). The third and fourth groups are separated by the first principal component (in the negative region) with no relevant contribution from the second principal component.

These results show only a partial separation for the honey samples according to the most predominant pollen and implies that the second, and even the third, predominant pollen are important for the classification procedure. However, the mixture of samples in the groups may be due to sensitivity of the sensor device to other honey components, rather than pollens, which are related to other factors that affect the honey characteristics such as soil composition, climate and others.

Linear discriminant analysis was also applied to the data in order to differentiate and classify the honey samples in three groups according to the most predominant pollens (*Lavandula*, *Erica* and *Echium*) present in the honey samples.

Globally, for the three honey groups, the 20 sensor signals showed normal distribution ($p > 0.010$ using the Kolmogorov–Smirnov with Lilliefors significance correction and Shapiro–Wilk tests). For the cases where normality was not achieved the skewness and the kurtosis of the distribution were investigated. The results showed that the signals obtained from the sensors present a symmetric and mesokurtic distribution (absolute value of skewness/standard error and kurtosis/standard error ratios lower than 1.96), except

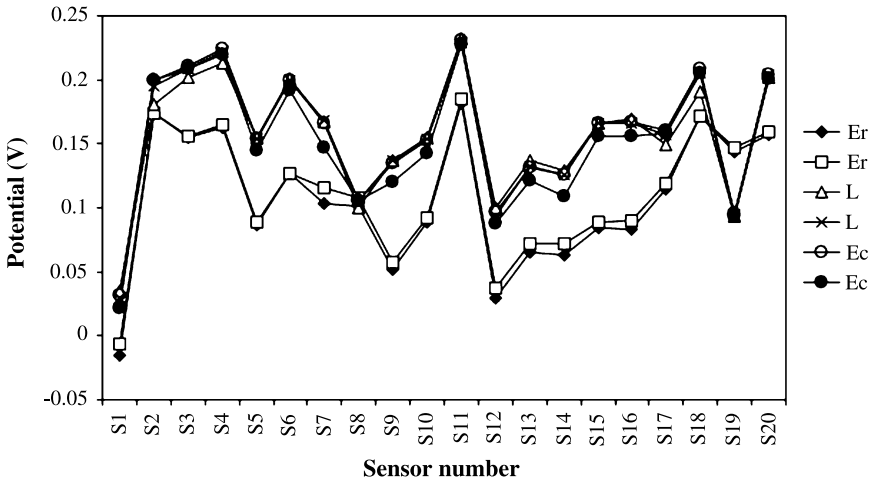

Fig. 2. Potential signal patterns for six honey samples – Predominant pollen: Er *Erica*; L *Lavandula*; Ec *Echium*

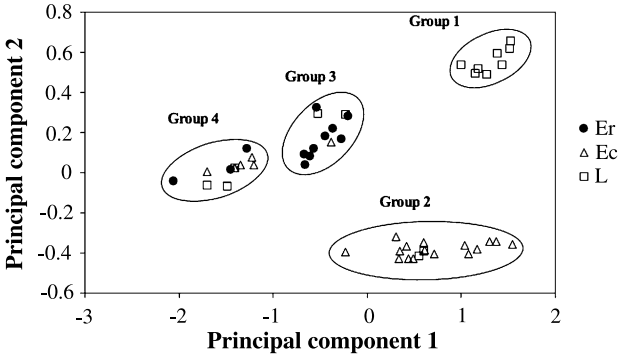

Fig. 3. Two-dimensional plot of the first two principal component scores of the signal profile – Predominant pollen: Er *Erica*; Ec *Echium*; L *Lavandula*

for sensors S3 and S19 for *Lavandula* honeys and sensor S18 for *Erica* honeys. Regarding the homogeneity of variances, the Levene test showed that in general the homogeneity assumption is not verified ($p < 0.013$). Since the normality assumption is not a problem and to overcome the lack of data homocedasticity, the one-way ANOVA with Welch's correction was performed for testing equality of means, showing that significant differences were found for all the independent variables ($p < 0.050$) except for sensor S8 ($p = 0.207$).

The discriminant analysis was performed using a minimum tolerance level of 0.010 to eliminate the variables that provide superfluous information. The variables failing the tolerance test were the sensors S16, S17 and S20. Two discriminant functions with acceptable prevision were established, as shown in Fig. 4, where the results for classification with the obtained discrimination model are presented. The Wilks' Lambda test showed that both the canonical discriminant functions were significant ($p < 0.006$).

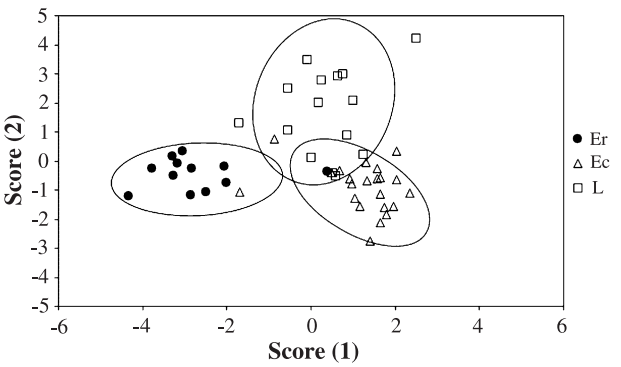

Fig. 4. Two-dimensional plot of the first and second discriminant function – Predominant pollen: Er *Erica*; Ec *Echium*; L *Lavandula*

The *Erica* pollen shows negative scores and *Echium* pollen shows positive scores for function 1, thus allowing the separation of these two kinds of samples. The *Lavandula* pollen scores are close to zero for function 1 and show positive values for function 2. The second discriminant function allows *Lavandula* pollen differentiation, whereas the *Erica* and *Echium* pollen have values close to zero. However, Fig. 4 shows that the discrimination was not perfect.

For the 50 honey samples analysed, linear discriminant analysis allowed to correctly classify 84% of the original data showing a satisfactory robustness since it allows the correct classification of 72% of the honey samples for cross-validation procedure. In this analysis, the honey samples correctly classified for the different types of predominant pollen were 53% for *Lavandula*, 83% for *Erica* and 78% for *Echium* pollen.

Conclusions

The results of this study show that the assembled electronic tongue allows reasonable, although not perfect, differentiation between honey samples accordingly to the most predominant pollen. Probably, the existence of a second classification pollen in large amounts in some of the honey samples analysed (samples with both pollens in high content: *Lavandula–Echium*, *Erica–Lavandula* and *Echium–Lavandula*) misleads the discrimination in some extent.

Further work involving a more complete characterization of honey samples by classical analysis (physicochemical, melissopalynological and organoleptic techniques) for confirmation of honey identification [13–15], may clarify the results obtained as response of the present electronic device. On the other hand, the influence of the degree of predominance of a pollen type (for instance, samples with pollen predominance higher than 80%) on the differentiation provided by the device deserves further investigation, although it is probably difficult to obtain samples with this characteristic in large numbers. Moreover, another point that deserves further investigation is the improvement of the tongue by replacement of some of the sensors by others more suitable for providing more diversified signals. More advanced classification methods, for example Artificial Neural Networks or Support Vector Machines, may be attempted to improve the obtained classification performance [20].

In conclusion, albeit not perfect, the present electronic tongue shows promising behaviour for

monofloral honey assortment as an alternative or complementary tool to the classical analytical methods for quality control of honey samples.

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