Rapid and Precise Discrimination of Wines by Means of an Electronic Nose Based on Gas-chromatography

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An electronic nose of the latest generation, based on the chromatographic principle, was used to analyze the volatile profile of various samples of wine in order to assess the possibility of differentiating wines based on various parameters such as their geographic origin, grapevine variety and its characteristics (such as noble/ hybrid character), the yeasts used for fermentation in the winemaking process, the proportions of different wines used in blends or even fraud attempts such as the dilution with water. It was found that the multivariate data analysis procedures such as the Discriminating Factor Analysis, applied to the chromatographic data recorded using the electronic nose, allows for a very good discrimination of the mentioned wine samples.

Keywords: wine, electronic nose, sample discrimination, multivariate statistics

Electronic noses are devices of various types and principles widely used as analytical tools in the food and drinks industry [1-4], as well as in the fields of medicines and chemicals. An electronic nose is used to detect volatile substances and consists of an array of sensors that colects chemical signals that are afterwards analysed and interpreted in a way that mimics the human nose evaluation, by using a pattern recognition method of data analysis. With any type of electronic nose it is possible to distinguish a sample from another or to determine the evolution in time of the state or condition of a sample, as the headspace from each sample has a unique volatile fingerprint which the device is able to record.

E-nose instruments are undergoing a continuous improvement. From the first devices in this category which were simple arrays of several sensors based on polymers and metal-oxide semi-conductors, each of them responding to the presence of one certain substance, in recent years modern and powerful apparata were developed based, for example, on gas chromatography. In this case we don't have a physical array of sensors, each of them detecting a certain substance; instead, the numerous chromatographic peaks in the chromatogram recorded by the apparatus is handled as the response of a "virtual" sensor [5]. Unlike in the conventional usage of the gas chromatograph, in this case it is not strictly necessary to know the significance of each chromatographic peak – in the same way as, when we smell a certain food product, we do not usually identify all the components of the aroma. In both cases, nose or electronic nose, the accent is rather on grasping the overall "volatile fingerprint" of the product. Just as the human nose is sometimes capable of differentiating two samples which are only slightly different, the modern electronic noses display great discrimination power. This is mainly due to two factors – the very good sensitivity (which means they are able to respond to many aromatic compunds, evidentiated as peaks in the chromatogram) and powerful procedures for data analysis, especially multivariate statistics (which allow for pattern recognition and identification of sample grouping tendencies).

Wines offer many opportunities for the use of an e-nose and in recent years there has been considerable interest for finding and refining ways for discriminating wine samples based on various parameters such as grapevine variety [6], geographic origin [4, 7, 8], vintage year [9] and wine aging [10], technological and experimental factors [11], detection and prediction of some sensory properties [12, 13], authenticity and tipicality etc. [14, 15].

This short research note presents briefly some of our results in these directions, obtained by means of a relatively new type of electronic nose constructed on the basis of a flash gas-chromatograph with two columns.

Experimental part

Materials and methods

The apparatus, Heracles Analyzer / Electronic Nose produced by Alpha MOS. (France), consists of a Gas Chromatograph with two columns, coupled with a Combi PAL Auto-Sampler System (made by CTC Analytics AG, Switzerland) which is useful for processing multiple samples in batches. The samples taken by the auto-sampler are sent via syringe to an injector where a flash evaporation process takes place. Gaseous compounds from the sample headspace are then passed to an adsorbent Tanax trap where they are concentrated, and then the system is heated causing the trapped compounds to be released and subsequently injected simultaneously to two capillary chromatographic columns (one apolar, GC#1: DB-5 and the other one with medium polarity, GC#2: DB-1701) and two flame ionization detectors (FID) working in parallel, thus providing two chromatograms which are recorded and later analyzed. The hydrogen used as carrier gas is FID Grade (Ultra High Purity, 99.999%). The usual working parameters for wine samples are: injected volume 2500 µL, measurement time 20 s, trap temperature 40°C, pre-purging time 5 s, trap preheating 20 s, trap baking 60 s, trap desorption temperature 250°C, column heating at 40°C and maintaining constant for 2 s up to 200°C with an increment of 5°C/s and 5 s maintaining at the final temperature, injection temperature 200°C, detector temperature 220°C and acquisition time 40 s.

The principle which basically transforms the Heracles analyzer into a powerful electronic nose is as follows: each of the recorded chromatographic peaks is regarded as the response of a virtual sensor, which measures a particular volatile component of the sample. As a result, each peak recorded for a certain wine can be interpreted as the value of a certain *variable* (or *dimension* in multivariate statistics)

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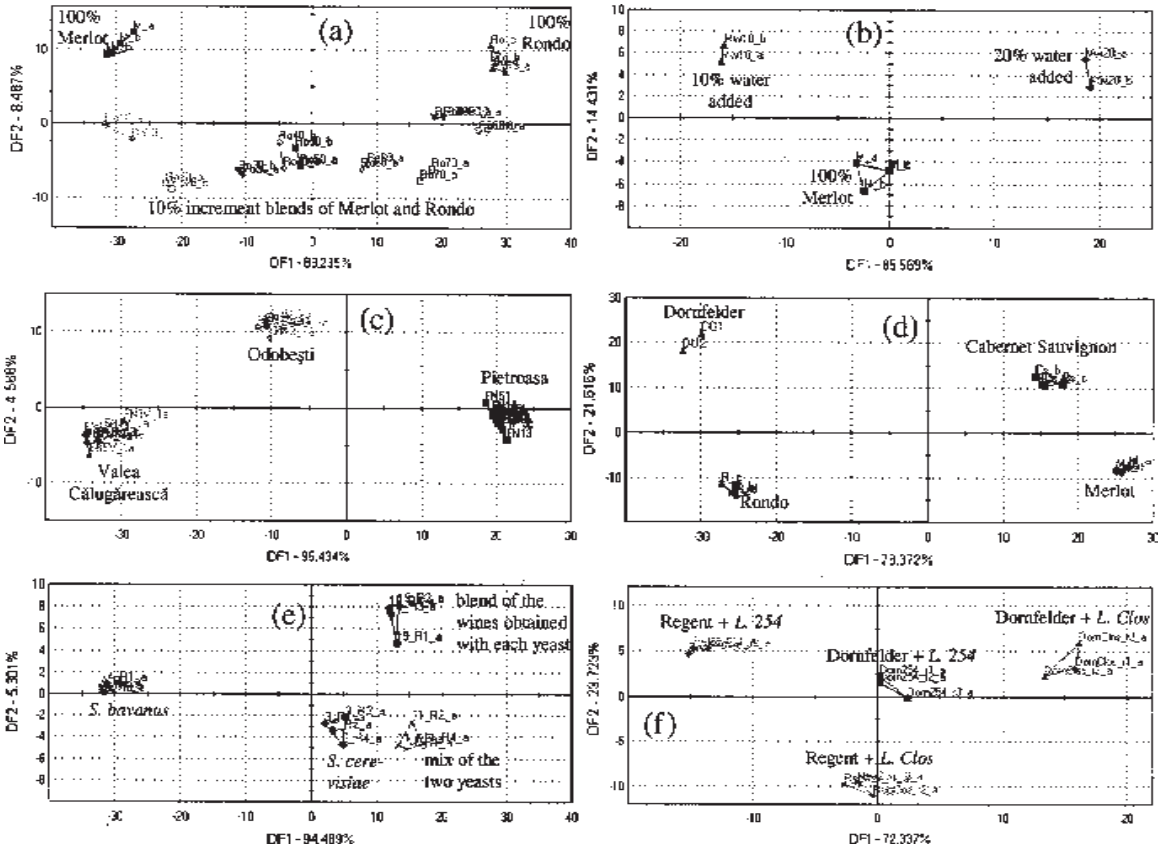

Fig. 1. Examples of differentiation of various types of wine samples using the electronic nose

for that particular wine. Since there are many peaks in the chromatogram, for a batch of sample wines a matrix of results is obtained, with *m* rows (*m*=number of samples) and *n* columns (*n* = the number of variables/peaks identified and measured). This matrix can then be analyzed using appropriate software – in our case, the software provided with the apparatus, Alpha Soft ver. 11.0, which offers various methods for comparing and classifying samples. Among these methods, Principal Component Analysis (PCA) and Discriminant Function Analysis (DFA) are most often used. Both PCA and DFA take the original sets of data and identify some linear combinations of the initial variables, combinations called Principal Components in case of PCA and Discriminant Factors in case of DFA, which allow a simpler representation of the initial data while preserving as much as possible the information contained by it. We found that the best discrimination among samples was obtained using DFA, which also provided some possibilities for identifying unknown samples. It should also pe mentioned that the DFA procedure as implemented by the Alpha Soft package of the apparatus performs an initial evaluation of the chromatographic peaks recorded and determines their degree of significance for the discrimination among samples. Therefore, very small peaks which barely exceed the noise level are disregarded and the final DFA analysis only takes into consideration a selection of peaks (virtual sensors) which are most efficient in ensuring the differentiation among samples.

Results and discussions

Figure 1 shows several examples of the differentiation of wine samples achieved by using the Heracles electronic nose. These plots are obtained by applying the DFA multivariate analysis procedure on the sets of data (retention times and peak height or area) obtained from the chromatograms provided for each sample by the two columns of the apparatus. No attempt was made to identify which specific volatile compound corresponds to each of the chromatographic peaks; instead, the accent was put on the capacity of the apparatus to differentiate various samples in the same way as the human nose does – by evaluating globally the overall aromatic profile, all of the information provided by the recorded gas chromatograms.

Plot (a) is a Discriminant Function Analysis (DFA) plot showing the differentiation by the electronic nose of two wines (Merlot and Rondo – samples located at the left and right of the diagram) as well as eight of their blends ranging from 90% Merlot + 10% Rondo, to 10% Merlot + 90% Rondo, in steps (increments) of 10%. As explained above, the DFA method identifies two Discriminant Factors called DF1 and DF2 which explain 89.24% and 8.47% of the variability in the initial data set, and which are represented as the axes of the DFA plot. The samples are then plotted in this space, and they tend to be grouped according to the degree of resemblance or difference in their data, which is directly related to the number and size of chromatographic peaks recorded by the electronic nose when each of these samples was analyzed. It thus becomes clear, from examining plot (a), that the apparatus is perfectly able to sense differences of 10% in the blending proportions of Merlot and Rondo. This result suggests that the apparatus and method can be used successfully for the identification of the wine variety (authenticity) and even the proportion of various varieties in a blend.

Plot (b) shows the differentiation of Merlot wine samples from samples of Merlot with 10% and 20% water addition. Obviously the addition of water leads to a dilution of the volatile compounds from the wine; the electronic nose is able achieve a good discrimination of the three types of samples. This result too has implications towards the

appropriateness of the method for the evaluation of wine authenticity as well as fraud detection.

Figure 1 (c) presents the separation in the DFA space of samples of Feteasca neagra wine obtained in 3 different vine growing regions (Odobeşti, Pietroasa, Valea Călugărească). It must be mentioned that within each of the 3 groups of samples there was wide variation regarding the winemaking technology (different enzyme treatment, maceration period, addition of color stabilizers, etc). Nevertheless, in spite of these variations, the electronic nose was able to group the samples and to differentiate them on the basis of the geographical origin of the grapes used for obtaining all these wines. This is a remarkable result which illustrates the very essence of the electronic nose: the capacity of extracting information from the overall evaluation of the aromatic profile, without the need to identify each of the components of this aromatic profile (a task which would be very difficult and time consuming in a situation like the one presented here). One single measurement of each sample is enough to be able to separate this large number of wines with so different technological characteristics – into three groups related to the area or origin.

Plot (d) shows the discrimination of samples of four red wines, of which two were made of noble varieties (Cabernet Sauvignon and Merlot) and two of hybrid varieties (Dornfelder and Rondo). In this case the apparatus achieves a double differentiation: first, all samples are well discriminated (separated in four distinct groups of wines); second, samples in each category (noble, hybrid) are also differentiated on the basis of variety. It is very interesting, again, that this differentiation – very important in the wine world, with implications on wine authenticity and value – is achieved by a single analysis of each sample, without the need for the identification and quantification of the many compounds which contribute to make the difference between noble and hybrid wines.

In plot (e) there is the e-nose differentiation of wines obtained using a yeast strain with killer character (*Saccharomyces cerevisiae* NT 116 Anchor), a strain of *S. bayanus* (AWRI 1176) and a mix of the two strains in equal proportions. There was also a blend (50%-50%) of the two wines fermented using each of the two strains alone. Again the electronic nose achieves a good discrimination of the four groups of samples.

Finally, plot (f) shows the double differentiation of wines of two varieties (Regent and Dornfelder) made using two yeast strains (Lalvin Clos and Lalvin 254), respectively. In other words the electronic nose was able to distinguish wine samples based on the variety and the yeast strain used for their fermentation.

Conclusions

With a good selection of the sensors (peaks) used for the multivariate analysis, samples belonging to various vine varieties or blends, originating from various regions or obtained with different winemaking techniques can be well discriminated with the e-nose.

This offers potentially numerous applications for the study of the wine authenticity: by plotting unknown samples together with known ones it is in principle possible to identify the grapevine variety, the place of origin, the yeast used in the fermentation process, the percentage of a variety in a blend or if a certain winemaking technique has been applied or not. Other applications can be imagined, such as the evaluation of wine quality by comparison of a sample with standards of known quality, or the study of the effects of variations and improvements in winemaking technologies (in which case wines produced in accordance with new technologies would be compared to those obtained by classic methods). By use of pattern recognition analysis techniques, the volatile profile generated by the e-nose and sensory attributes measured with human panelists could lead, for some applications, to the replacement of the later with the rapid and convenient GC-e-nose techniques.

References

1. SANTOS J. P., LOZANO J., ALEIXANDRE M., ARROYO T., CABELLOS J. M., GIL M. and HORRILLO M. C., 2010, Threshold detection of aromatic compounds in wine with an electronic nose and a human sensory panel, *Talanta*, Volume **80**, Issue 5, p. 1899
2. ESPOSTO S., MONTEDORO G. F., SELVAGGINI R., RICC I., TATICCHI A., URBANI S. and SERVILI M., 2009, Monitoring of virgin olive oil volatile compounds evolution during olive malaxation by an array of metal oxide sensors, Food Chemistry, Volume **113**, Issue 1, p. 345
3. LUYKX D.M.A.M., VAN RUTH S.M, 2008, An overview of analytical methods for determining the geographical origin of food, Food Chemistry, Volume **107**, Pp. 897
4. SANTOS J.P., ARROYO T., ALEIXANDRE M., LOZANO J., SAYAGO I., GARCÍA M., M.J. FERNÁNDEZ, ARÉS L., GUTIÉRREZ J., CABELLOS J.M., GIL M., and HORRILLO M.C., 2004, A comparative study of sensor array and GC-MS: application to Madrid wines characterization, Sensors and Actuators B: Chemical, Volume **102,** p. 299
5. ALPHA MOS, 2002, Technical Note N-DT-01: Statistical Analysis, released October 2002, p. 1
6. DI NATALE C., DAVIDE F.A.M, D'AMICO A., NELLI P., GROPPELLI S., and SBERVEGLIERI G., 1996, An electronic nose for the recognition of the vineyard of a red wine, Sensors and Actuators B: Chemical Volume **33**, p. 83
7. BURATTI S., BENEDETTI S., SCAMPICCHIO M., PANGEROD E., 2004, Characterization and classification of Italian barbera wines by using an electronic nose and an amperometric electronic tongue, Analytica Chimica Acta, **525**, p. 133
8. COZZOLINO, D., SMYTH H.E., LATTEY K.A., CYNKAR W., JANIK L., DAMBERGS R. G., FRANCIS I. L. and GISHEN M., 2006, Combining mass spectrometry based electronic nose, visible-near infrared spectroscopy and chemometrics to assess the sensory properties of Australian Riesling wines, Analytica Chimica Acta, v. 563 , 2006, p. 319
9. GARCÍA M., ALEIXANDRE M., GUTIÉRREZ J., HORRILLO J. and HORRILLO M.C., 2006, Electronic nose for wine discrimination. Sensors and Actuators B: Chemical, Voume **113**, p. 911
10. LOZANO J., ARROYO T., SANTOS J.P., CABELLOS J.M. and HORRILLO M.C., 2008, Electronic nose for wine ageing detection, Sensors and Actuators B: Chemical, Volume **133**, Issue 1, p. 180
11. CHATONNET, P. and DUBOURDIEU D., 1999, Using electronic odor sensors to discriminate among oak barrel toasting levels, J. Agric. Food Chem, **47**, p. 4319
12. BURATTI S., BALLABIO D., BENEDET S., COSIO M.S., 2007, Prediction of Italian red wine sensorial descriptors from electronic nosehotometric measurements by means of Genetic Algorithms regression models, Food Chemistry, **100**, p. 211
13. LOZANO J., SANTOS J.P., GUTIÉRREZ J. and HORRILLO M.C., 2007, Comparative study of sampling systems combined with gas sensors for wine discrimination, Sensors and Actuators B: Chemical, Volume **126**, Issue 2, p. 616
14.ALEIXANDRE M., LOZANO J., GUTIÉRREZ J., SAYAGO I., FERNÁNDEZ M. J. and HORRILLO M. C., 2008, Portable e-nose to classify different kinds of wine, Sens. Actuators B: Chem., **131**, 71
15. PERIS M. and ESCUDER-GILABERT L., 2009, A 21st Century Technique for Food Control: Electronic Noses, Analytica Chimica Acta, **638**, p. 1

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