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Article

Rapid and Non-Destructive Analysis of Corky Off-Flavors in Natural Cork Stoppers by A Wireless and Portable Electronic Nose

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Abstract: This article discusses the use of a hand held electronic nose to obtain information on the presence of some aromatic defects in natural cork stoppers, such as haloanisoles, alkylmethoxypyrazines and ketones. The proposed prototype has been developed as an instrumentation system with up to eight commercial gas sensors. Machine learning algorithms such as k-nearest neighbors and artificial neural networks has been used. The use of this system tries to improve the current aromatic defect detection process in the cork stopper industry, which is done by gas chromatography or human test panels.

Keywords: Natural cork stoppers; Corky off-flavors; Electronic nose; Machine learning algorithms; Artificial neural networks

1. Introduction

Cork stoppers are the main closures chosen to seal the wine bottles, and will influence the quality of the wine, mainly those that require long stays in the bottle. For this reason, the cork industry has strict quality systems, the goal of which is the total absence of defects. In this sense, one of the main problems of the cork industry is the detection of the defect known as "cork taint".

Halogenated aromatic compounds have been identified as the typical cause of the "cork taint" defect, specifically the 2,4,6-tricloroanisol (TCA) and, to a lesser extent, 2,4,6-tribromoanisol (TBA) and 2,3,4,6-tetracloroanisol (TeCA) [1,2]. However, apart from these halogenated derivatives, cork present other volatile compounds with negative effects such as geosmin, with a strong smell of mold and wet earth, guaiacol with phenolic olfactory notes, 1-octen-3-ol and 1-octen-3-one, with a strong mushroom and earthy odor, or 2-methoxy-3,5-dimethylpyrazine (MDMP) with a characteristic musty and moldy odor [3-5].

Most of the cork industries have a gas chromatography system coupled with mass spectrometry, which allows the quantification of different corky off-flavors in cork stoppers. This technique needs a previous step of sample preparation, usually destructive and time-consuming, that sometimes requires the use of organic solvents, such as purge and trap, solid phase microextraction, soxhlet extraction, stir bar sorption or pressurized fluids [5-7].

The control of the presence of typical and atypical compounds responsible of “cork taint” by chromatographic techniques is very useful, although due to their time-consuming and destructive character, only can be applied to a representative sample of cork stoppers and cannot be included in the production lines. Also, some industries have a system

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for detecting olfactory defects by the human sense, with a great degree of subjectivity. In this sense, it is of special interest to introduce in the quality control of the cork industry a non-destructive analytical technique of the aroma that allows the rapid and precise analysis of many cork samples in a relatively short time. Among the available techniques, the electronic nose (e-nose) stands out. An e-nose is an instrument composed of a set of chemical gas sensors with partial specificity and an appropriate pattern recognition system, capable of recognizing simple or complex odors [8]. These sensors can be used for the analysis of volatile compounds in different matrices, by transducing chemical signals into electrical signals [9]. In the bibliography there are several studies about the use of this analytical tool in the wine industry, including aging control, wine discrimination or grape ripening monitoring [10-12]. However, there are practically no studies on their use in the cork industry. It is worth highlighting the determination of TCA in wines using a coupled system of headspace and mass spectrometry [13], or the use of a miniaturized sensor module to detect TCA in cork stoppers [14].

In this work, the feasibility of a small wireless and portable e-nose (WiNOSE 6), capable of measuring up to eight microsensors, to detect typical and atypical off-flavor compounds in natural cork stoppers, has been studied. The selected compounds belong to three different chemical families, haloanisoles, alkylmethoxypyrazines and ketones, TCA, MDMP and 1-octen-3-one, and they present a strong musty, moldy and earthy odor.

2. Materials and Methods

2.1 Cork samples

Flower quality natural cork stoppers without sensorial deviant odors were kindly provided by Gruart La Mancha S.A. (Valdepeñas, Ciudad Real, Spain). The cork stoppers became contaminated, by triplicate, with increasing amounts (5, 15, 30 and 60 ng per cork) of 2,4,6-thichoroanisole (TCA) (Merck KGaA, Darmstadt, Germany), 2-methoxy-3,5-dimethylpyrazine (MDMP) (Enamine Ltd., Kyiv, Ukraine) and 1-octen-3-one (Merck KGaA, Darmstadt, Germany). In the case of 1-octen-3-one, the cork stoppers became contaminated with 5, 15, 30, 60 and 120 ng per cork. Once the cork stoppers were contaminated, they were stored under vacuum until their analysis.

2.2 Electronic nose

A schematic and a photograph of the e-nose used are shown in Fig. 1.

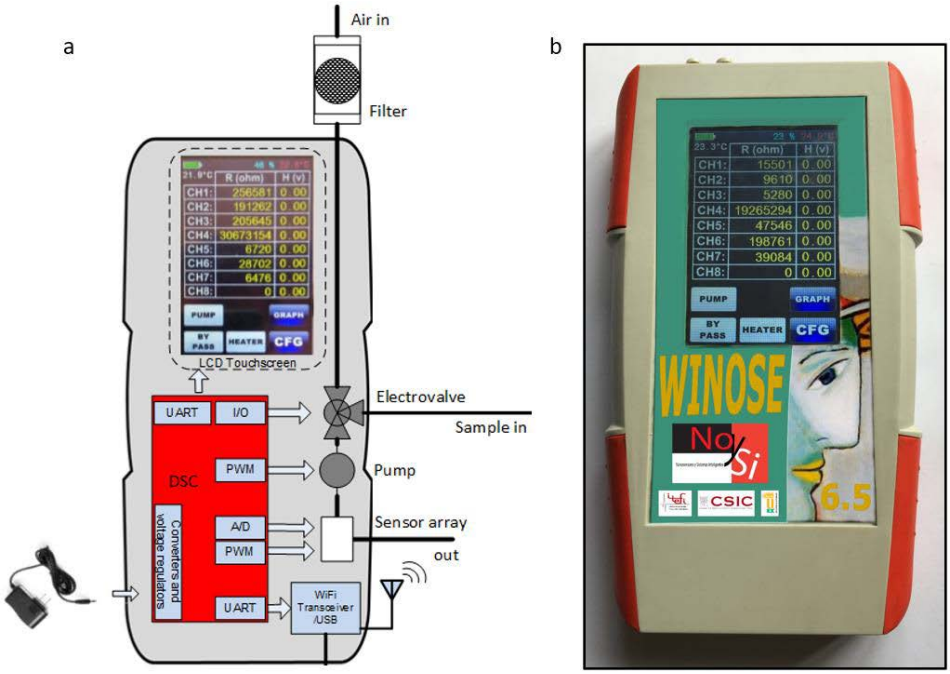

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Figure 1. E-nose; a) Schematic, b) photograph.

The e-nose is a compact size and light instrument that has the possibility to measure up to eight commercial microsensors. It has a micropump to create a flow through the sensor chamber and an electrovalve to choose the sampling path. It also has an internal temperature and humidity sensor and the possibility to connect and measure an external temperature sensor. Different arrays of sensors are available to be used with this prototype. For this work, we used two different arrays from several manufacturers: MICS (SGX Sensortech, Bern, Switzerland), CCS (AMS, Premstaetten, Austria) and TGS (Figaro Engineering Inc, Osaka, Japan). Table 1 shows the composition of the arrays of sensors used in the experiments. The sensors have integrated heaters, capable of reaching 500 °C with low power consumption (typically from 10 to 80 mW).

Table 1. Sensor setup.

Set 1	Set 2
MICS–2714	CCS801
MICS–5524	CCS803
MICS–4514–OX	MICS–4514–OX
MICS–4514–RED	MICS–4514–RED
MICS–5914	TGS8100
MICS–6814–OX	MICS–6814–OX
MICS–6814–RED	MICS–6814–RED
MICS–6814–NH3	MICS–6814–NH3

The instrument can be controlled using the touch screen or remotely by a software tool developed in LabVIEW (version 18, National Instruments, Austin, TX, USA). The program also displays and controls the measurement parameters (sensor resistance and heater values, ambient temperature and humidity, valve status, battery status, pump power).

2.3. Measurement protocol

Corks were introduced in 50 mL vials with two orifices in the top, one for atmospheric air and the other connected to the e-nose. Each measurement cycle consists in a desorption phase of 9 min followed by an adsorption phase of 1 min. For each sample cycles are repeated several times. Two groups of experiments have been carried out. The first one involves the measurements of all defect concentrations whereas in the second one only the maximum defect concentrations have been measured. The main difference is that in the first case, data processing has been performed offline and in the second case the data processing was performed online.

In the offline experiment 8 measurement were performed for each sample while in the online experiment 15 measurements were obtained for each sample.

The sensor responses were calculated as the relation between the equilibrium resistance value in air, Ra, and the equilibrium resistance value in the presence of the sample Rs:

$$r = Ra/Rs \quad (1)$$

2.4 Data processing

Several multivariate data processing techniques have been used: a linear unsupervised one, principal component analysis (PCA) and nonlinear supervised K-nearest neighbors (kNN) [15] and two types of artificial neural networks: multilayer feed forward

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neural network (MLFF) [16] and Radial basis neural networks (RBF) [17]. Principal component analysis is a chemometric linear, unsupervised and pattern recognition technique used for analyzing and reducing the dimensionality of numerical datasets in a multivariate problem. This method extracts the features from a data matrix in terms of a complementary set of scores (coordinates of the data in the new base) and loadings (contribution of the sensors to the components) plots. This method applies a linear transformation to the data and result in a new space of variables called principal components. The scores plot is usually used for studying the distribution of the data clusters. In the loading plot, sensors with similar contributions will be close together. Sensors close to the origin have comparably small contribution. The scores of the three first principal components were used in the training of the neural networks.

As we did not have many measurements leave one out cross validation was applied to check the performance of the network [15]. This method consists of training N distinct nets (in this case, N is number of measurements) by using N − 1 training vectors, while the validation of the trained net is carried out by using the remaining vector, excluded from the training set. This procedure is repeated N times until all vectors are validated. In the offline experiment PCA was performed by OriginPro (2019 version, OriginLab Corporation, Northampton, MA, USA) and kNN, RBF and MLFF were performed in Matlab (version 12, Mathworks Inc., Natick, MA, USA).

On the online experiment all the classification algorithms were written in Labview using the Machine Learning Toolkit (MLT) [18].

3. Results

After feature extraction using equation 1 data were preprocessed before PCA analysis. The preprocessing involved autoscaling and centering:

$$r_i = (r - \overline{r_i})/\sigma_i \quad (2)$$

where $\overline{r_i}$ is the mean and σ_i is the standard deviation of sensor i response over the input data. The distribution of values for each sensor across the entire database is set to have zero mean and unit standard deviation. The first three principal components accounted for more than 99 % of the cumulative variance. Ellipses in the score plots are drawn assuming a Gaussian data distribution and represent the curve with a 90 % probability.

Fig. 2 to 4 show the PCA score plots of the two principal components for the three defects.

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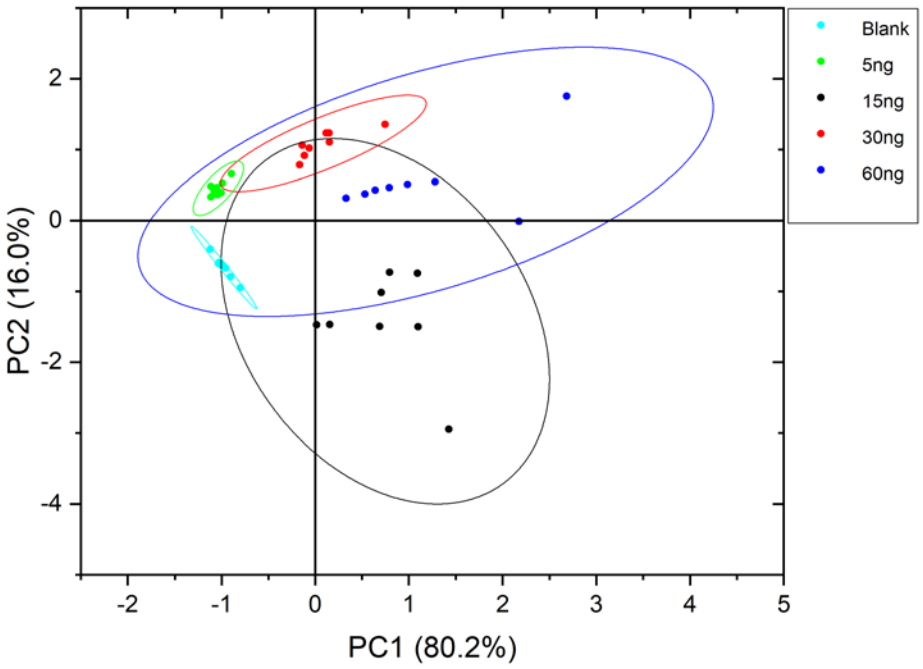

Fig. 2. PCA score plot for MDMP.

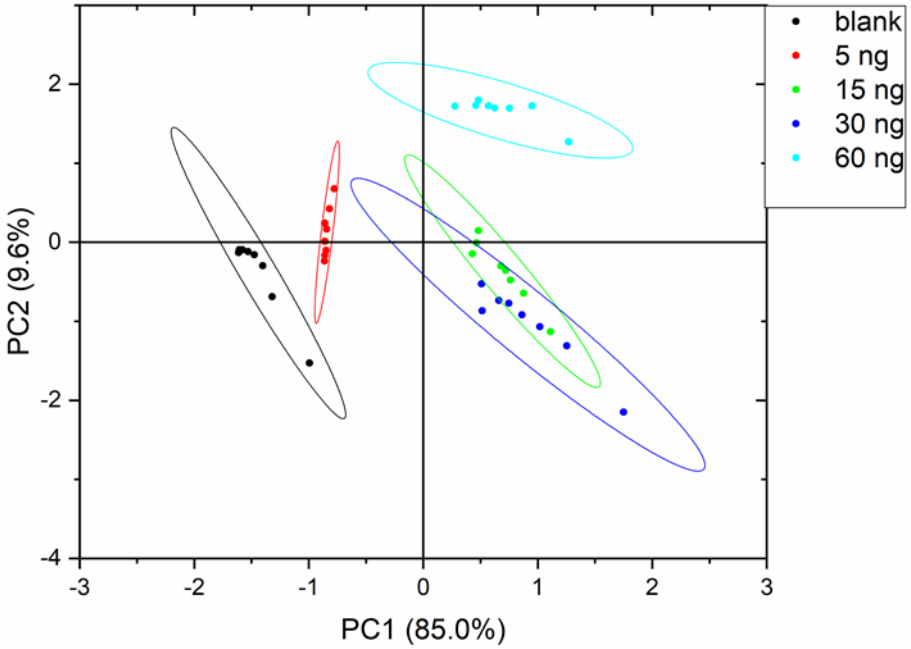

Fig. 3. PCA score plot for TCA.

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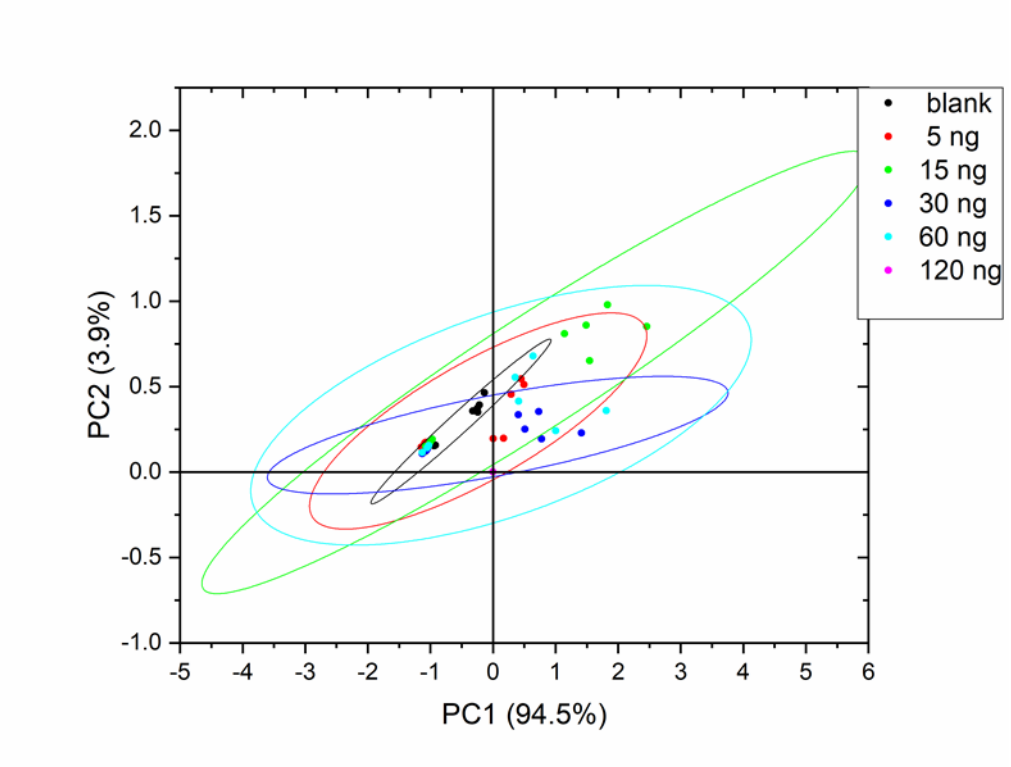

Fig. 4. PCA score plot for 1-octen-3-one.

From the above plots a better separation of classes were obtained for MDMP and TCA.

In general, the output of the neural network is given as a confusion matrix. From the confusion matrix a success rate is defined as the number of correct classified measures in each class over the total number of measures in this class. In our case, we have two success rates: a qualitative success rate and a quantitative success rate. In the quantitative case, each defect concentration is considered a separated class. In the qualitative case, we have only two classes: clean cork and cork with defects.

3.1 Offline experiments

The success rate are shown on Table 2

Table 2. Success rate for the different pattern recognition techniques for the offline experiments.

Defect	Qualitative			Quantitative		
	kNN	MLFF	RBF	kNN	MLFF	RBF
DMMP	100	100	100	98	100	100
TCA	98	100	100	82	100	98
1-octen-3 ona	90	97	97	*49*	77	54
All	85	99	99	*50*	98	95

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3.2 Online experiments

In this experiment, the e-nose uses the set 2 sensor configuration. Only the maximum concentration of each compound is measured. In this case, we have enough measurements to split the data in train + validation and test. The multivariate data processing techniques have been developed in Labview in order to integrate the measurement, control and data processing in one program. The qualitative success rate is 100 % for all techniques. The quantitative success rate is shown on Table 3.

Table 3. Success rate for the different pattern recognition techniques for the online experiments.

Method	Success rate
kNN	96
MLFF	99
RBF	98

As can be seen in the table above MLFF is the best classification technique although the other two give also an excellence performance. Once selected the best technique for the application the system is ready to perform measurement classification in real time. In order to test the performance of the system we made a simplified triangle test which is a discriminative method used in sensory science to assess if an overall difference is present between two products [21]. In this test, we used the blank sample (B) and the TCA sample (T) The e-nose measured the samples in the following order: BBT, BTB and TBB. The system gave the correct answer for all the sequences.

The results demonstrate the ability of the e-nose used to differentiate natural corks contaminated with different concentrations of TCA or MDMP, even as low as 5.0 ng. This is especially interesting since both compounds are the main responsible for the olfactory defect in wines called "cork taint". MDMP has a low odor threshold (2.1 ng L-1 in white wine) and a great affinity for wine, even higher than TCA, with a detection threshold of 4.3 ng L-1 in white wine [4, 5, 19]. In the case of MDMP, it is considered a very low risk of wine contamination if its concentration in the cork is less than 5.0 ng [5]. While for TCA, a mean percentage of migration from cork to wine of 4.7 % [20] has been estimated, which shows that the e-nose can detect TCA in corks at concentrations below those that could cause olfactory defects in wines.

4. Conclusions

The nose used in this work, composed of non-specific cross-sensitivity sensors that respond to a wide variety of compounds, can be applied in the wine industry to provide qualitative information about the sample and predict or detect the presence of cork-associated anomalies. Electronic nose has many advantages over traditional methods (gas chromatography, test panels). In addition, this sensory method could easily be applied to other beverages where cork odors or other off-flavors might be present. The system presented here has demonstrated to be very useful in the detection of aromatic defects in natural cork stoppers. We obtained near 100 % classification rates in the discrimination of defects such as MDMP, TCA and 1-octen-3-one. Real time sample classification has been developed with this prototype.

Electronic noses will probably not completely replace complex analytical instruments, but it offers fast real time detection and discrimination solutions and opens the way for their adaptation and integration into the internet of things.

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