

Artificial Diagnosis of Sensory Taints Due to *Brettanomyces spp.* Contamination in Valpolicella Wines

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Diagnosis and intervention to avoid Brett taints in the product can be a time-consuming task for the enologist in large production facilities and an instrumental and automated detection systems assisting the local expert technician would be desirable. This paper investigates whether electronic noses, which have been tested in other wine making and classification tasks, can be of use in detecting Brett taints in Valpolicella wines.

1. Introduction

Real-time in-process reference to a consolidated standard during sparkling wine production would be beneficial for reducing product loss and/or allowing a timely diagnosis of intervention needs (correction etc.). Periodic (but sometimes only end-point) control by assaying by the oenologist supervising batch production and a batch of chemical and physical analyses are normally carried out in wineries. Afterwards, each production batch must be sampled by the producer and must succeed the evaluation step on the institutional side before being legally labellable under the respective denomination. The institutional evaluation comprises a well-defined set of chemical and physical analyses regarding the parameters on which (legal or disciplinary) limits exist and a Commission assay. The Commission assay is only passed if the sample is approved at the analytical assay.

Marketing and technological needs motivate an instrumental monitoring of increased tightness. The consumer increasingly requires high sensory quality product and International markets require large amounts of highly standardised products, where normal batch-to-batch variations which would be denoted as 'typical' in the domestic market, are not acceptable. In order to save production time, defect rejection should begin with the early detection of non compliant batches.

Artificial sensory analysis is an oxymoron, given that equipment does not have senses; however, equipment that feature a limited number of non-specific sensor mimik Nature's senses which derives a large number of sensations from a limited number of receptors. Therefore, the sensor mimics the receptor, while a mathematical algorithm mimics brain in recognition and judgement. The researcher should complement the electronic nose (e-nose) sensors with an algorithm and adaption options capable of optimising the recognition capabilities of the system when it is used by an unexperienced operator.

E-noses have found two main fields of application: environmental monitoring (for environmental quality assurance, investigation, and liability; Dentoni *et al.*, 2012, Amodio *et al.*, 2012) and food product characterisation (Peris and Escuder-Gilabert, 2009); in this latter domain inter- (Alexandre *et al.*, 2009) and intra-varietal (López de Lerma *et al.*, 2013) identification, and real-time process monitoring (Pinheiro *et al.*, 2002; Lozano *et al.*, 2014). In winemaking, on-line fermentation monitoring has been proposed by density measurement, ethanol concentration and CO₂ evolution. Attempts have been made at the on-line measurement of specific by-products (e.g. by biosensors) and quality markers or of several simultaneous

products (by FT-IR or E-noses). This objective is highly ambitious, but not unrealistic (Sablayrolles, 2009). However, to the best of the authors' knowledge, no significant implementations of such a system has ever been established in any large scale facility as a production aid tool.

The present study has been carried out to assess whether the responses of an "electronic nose", i.e. a non-specific, gas-phase analytical instrument, is capable to draw an outline of the sensory profile of a red wine that has developed a taint after contamination by *Brettanomyces* yeasts. Contamination by *Brettanomyces* (e.g., *B. intermedius*) yeasts has always been considered detrimental to wine quality because it imparts to wine a pronounced animal-like odor taint ('Brett' in jargon). *Brettanomyces* sp. is capable of producing ethyl-4-phenol and ethyl-4-guaiacol from the hydroxycinnamic acids (*p*-coumaric and ferulic) in grapes but also develop in anaerobiosis in red wine after alcoholic fermentation is completed, consuming trace amounts of sugars that have been incompletely or not fermented by *S. cerevisiae* and producing large quantities of ethyl-phenols. These contaminations can also occur in the bottle (Ribéreau-Gayon *et al.*, 2006).

Valpolicella wines, just as other important red wines, may experience this contamination because of their manufacturing procedure, which includes: extended aging time (months to years); contact surfaces not properly sanitized, like the wooden staves of the barrels; aging technologies using micro dosage of oxygen. In cases where the preventive measures carried out by the technicians have proved ineffective, Brett taint requires the oenologist to intervene and perform a correction to salvage the batch. This task can be time-consuming in large production facilities, where as automatic pre-screening and early detection systems could assist the local expert technician.

Electronic noses have undergone extensive testing, and application, in environmental monitoring and food product characterisation. In winemaking, on-line fermentation monitoring has been proposed by density measurement, ethanol concentration and CO₂ evolution and, by the authors of this manuscript, likeliness of non conformity prediction for sparkling wines belonging to the "Prosecco DOC" and the Prosecco Superiore DOPG protected denominations (Franceschi *et al.*, 2015).

In this work, we tested a technique based on a quartz microbalance electronic nose to diagnose the presence of the Brett taint in Valpolicella wine. The developed procedure involved regular (i.e., non tainted) Valpolicella wine, tainted Valpolicella wine and the pure chemicals ethyl-4-phenol and ethyl-4-guaiacol. Purpose of this research was establishing the reliability of diagnosing the taint independently of the underlying wine character.

2. Materials and Methods

2.1 Experimental Design

Tainted and untainted Valpolicella wine supplied by the Collis group (<http://www.collisgroup.it/>) was analysed for its content of 4-ethylphenol (4EP) e 4-ethylguaiacol (4EG). The tainted batch was found to contain 4EP (1.9 mg/L) and 4EG (0.16 mg/L), while the content of 4EP and 4EG in the untainted batch was negligible. Accordingly, the following experimental design was devised. Independent, replicated (at least 3) and randomised measurements were carried out on the samples obtained from: 1. untainted wine, as such; 2. tainted wine, as such; 3. Untainted wine, contaminated by 4EP and 4EG at concentrations equal to the respective measured concentrations in the tainted wine; 4. Pure water, tainted by 4EP and 4EG at concentrations equal to the respective measured concentrations in the tainted wine; 5. ethanol/water mixture (12.5 % v/v as both tainted and untainted wine), tainted by 4EP and 4EG at concentrations equal to the respective measured concentrations in the tainted wine; 6. untainted ethanol/water mixture (12.5 % v/v); 7. water; 8. carrier gas. It should be noted that samples of group 3 were prepared in order to show whether naturally (i.e., during in-plant processing) and artificially tainted wine (i.e., untainted wine chemically contaminated after uncorking the bottle) show detectable differences from one another.

2.2 Experimental Setup

The artificial sensory analyses were carried out in a stream of pure nitrogen (cylinder), which was used both as a carrier stream (brushing the free surface of the sample in an Erlenmeyer flask), and as the reference substance (Figure 1 and 2). In order to avoid the stripping of aromas and volatile taints a special setup was devised permitting the full recycle of the equilibrated gas mixture stream (carrier gas + volatiles) during the measurement part of the overall sample analysis management procedure and avoid errors due to the coupling of desorption kinetics and the duration of the measurement. The three-way valve has two positions: 1-2 and 2-3. 1-2 is used during the loading of the carrier gas; the gas previously filling the circuit is discharged through the hydraulic guard. 2-3 is used during the active phase of the measurement. During this phase the gas is recirculated from the outlet port of the electronic nose to the sampler and back to the electronic nose inlet port. During the analysis phase of the carrier gas, which is also used as the reference standard stream, the gas sampled from the carrier gas line leaves the circuit through the hydraulic guard. An excess of carrier gas, signalled by a continuous leakage from the specific outlet port, avoids an opposite leakage of ambient air into the measurement circuit.

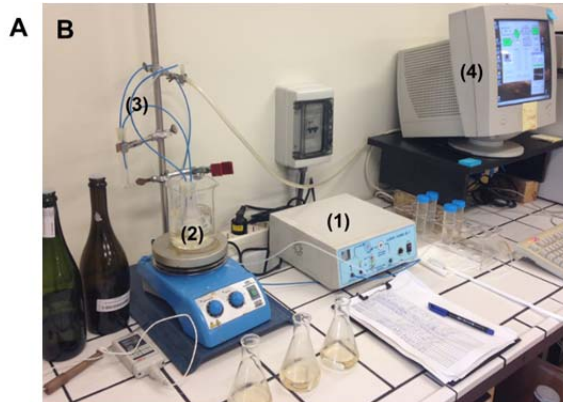
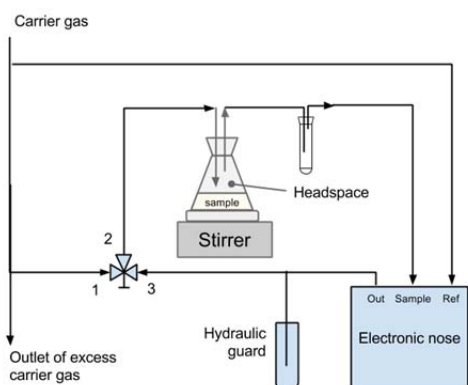


Figure 1: (A) Scheme of the measuring set-up including the sample holder, the piping, mode switching valves and guards. (B) View of the electronic nose sparkling wine sample assaying setup showing: 1. the E-nose; 2. the sample assaying chamber; 3. the carrier gas and mixture gas piping and the ancillary gas management devices; 4. the personal computer deputed to E-nose control.

The electronic nose used throughout the experimentation was a Libra Nose rev. 2.1, featuring multiple quartz micro-balance technology by Tor Vergata University of Roma. The steady state readings of the 8 E-nose sensors on the reference gas were subtracted to the sensor response to the reading with the sample wine. Following that, the deviations of the steady state readings obtained on the relevant sample obtaining the raw data ready for subsequent chemometric analysis. The steady state value of the measured relative humidity of the circulating sample was added to the 8-dimension array of sensor data so that each experimental run was described by a 9-number array.

2.3 Chemometric Analysis

Classification was carried out in non supervised and supervised manner by using Octave (<http://www.gnu.org/software/octave/>) on an Ubuntu Linux personal computer.

Prior to data analysis, the whole data set was standardised, that is, each variable (e.g., relative humidity and sensor 1 to eight) was mean centered and standardised (e.g., transformed into unit variance). Then, non supervised classification was carried by Principal Component Analysis by retaining two principal components, mindfully selected as described in the Results section.

The main goal of a Principal Component Analysis (PCA) is to identify patterns in data: finding the directions of maximum variance in high-dimensional data and project it onto a smaller dimensional subspace while retaining most of the information. The whole dataset consisting of d -dimensional samples ignoring the class labels was used to compute the 9-dimensional mean vector and the covariance matrix of the whole data set. Then, the eigenvectors and corresponding eigenvalues were computed, and the eigenvectors were sorted by decreasing eigenvalues. The 2 eigenvectors with the largest eigenvalues were taken to form a 9×2 dimensional matrix W and this eigenvector matrix was used to transform the samples onto the new subspace. This can be summarized by the mathematical equation $y = W^T x$, where x is a 9×1 -dimensional vector representing one sample, and y is the transformed 2×1 -dimensional sample in the new subspace.

3. Results and Discussion

Preliminary data inspection showed that relative humidity (RH) affects all sensor measurements. Therefore, RH was included in the measurements together with the sensor readings. Following that, principal components were calculated and components 1 and 2 were chosen. Figures 2 and 3 report the loading plot and the score plot respectively. The score plot shows a central area occupied by promptly analysed (on day 0 after the opening of the bottle) untainted wine, a North-East area occupied by promptly analysed tainted wine, a Southern area occupied by promptly analysed, artificially contaminated wine and a South-East area occupied by a dense, intermixed plot of samples belonging to all three sample categories analysed on subsequent days (1 and 2 days after the opening of the bottle).

Prompted by the scarce clarity of the classification map in the South-East dense area we investigated closer the time evolution of the samples. The time evolutions of the average between replicate of sensors 1 and 3 signals are shown in Figure 4 as an example: it is readily seen that the sensor signals tend to gather together in the 1-day-old samples, while they fall apart again after 2 days. We decided therefore to investigate the significance of the individual sensor collected data and carried out a 1-way ANOVA of each sensor data after

0, 1, and 2 days. Table 1 reports the ANOVA of sensor data, which clearly shows that the data collected by analysing the sample material right after opening the wine bottle is mostly significant at the p-value of 0.05, while it is not anymore 1 day after. Subsequently, significance is mostly recovered; however, these data should not be used for multiple reasons: 1. the sensor signal variation appears to be smaller, hence the detection may be less accurate, 2. this change may occur on a slower time scale, so that non significant data might be incorrectly accepted for drawing a totally inaccurate conclusion, 3. To be really useful the overall analysis + detection process should be carried out at the earliest. For coherence, the data obtained from sensor 8 were then excluded from the analysis, and the PCA was re-done. Indeed, the classification map reported in Figure 3 actually refers to this latter, final, case (one sensor datum excluded) and was referred to previously because the complete sensor data map did not differ from this latter sufficiently to warrant the inclusion of a further figure. Obviously, this figure still shows the confusion among sample sets referring to subsequent days which supports the suggestion that only freshly analysed samples may be used to draw conclusions about Brett contaminations.

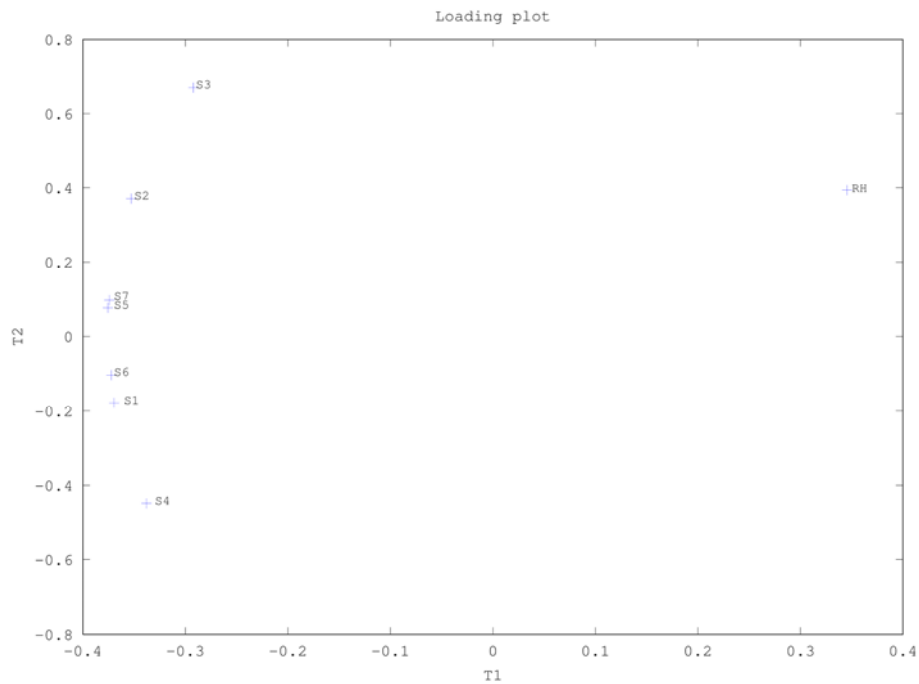


Figure 2: Loading plot of the results of the performed PCA analysis.

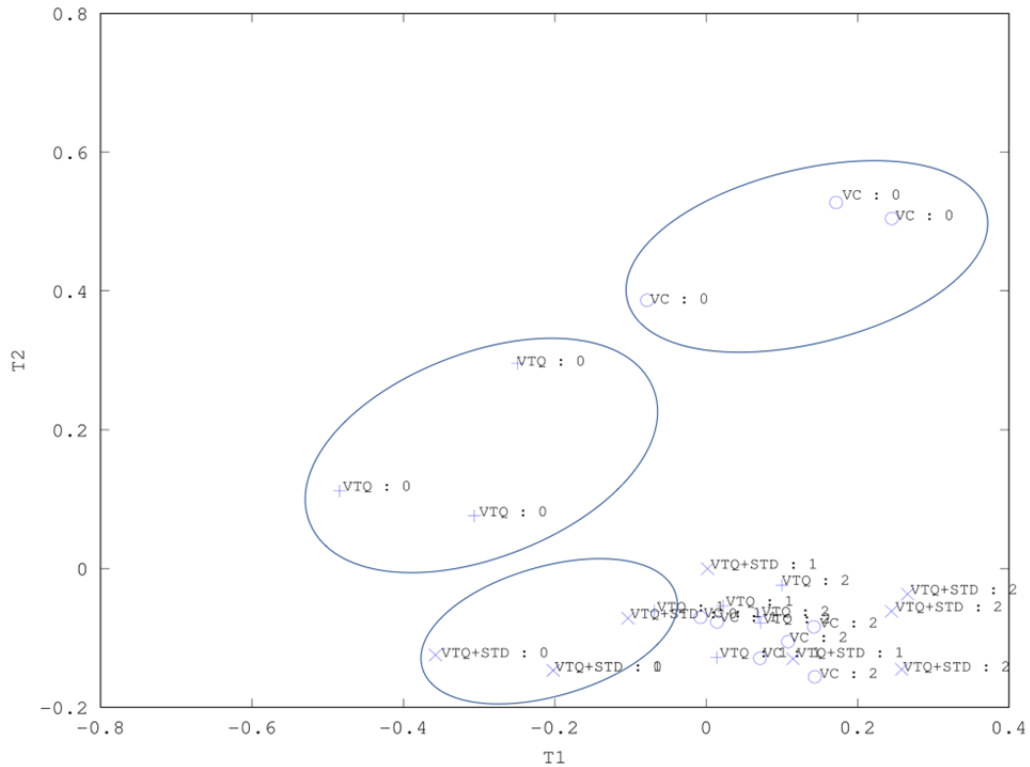


Figure 3: Score plot of the results of the performed analysis. Label coding indicates <sample type> : <aging>. Sample types coding is as follows: VTQ: untainted wine; VC: tainted wine; VTQ+STD: artificially tainted wine; STDETA: tainted water: ethanol mixture; STDH2O: tainted water. Aging indicates the time past (in days) after the first measurement.

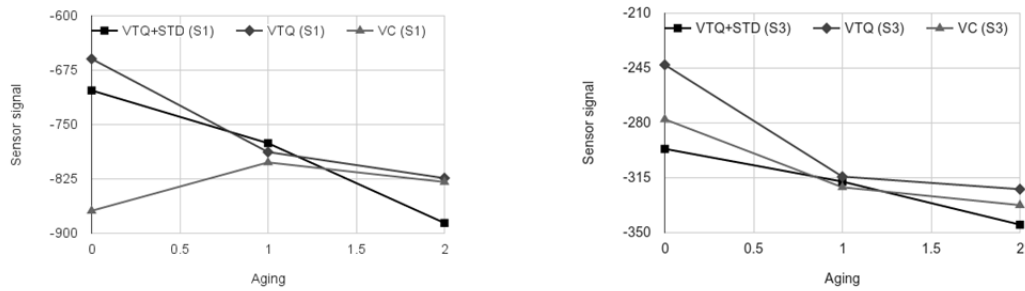


Figure 4: Time evolution of individual electronic nose sensor detection of untainted wine (VTQ), synthetically tainted wine (VTQ+STD) and naturally tainted wine (VC) of sensor 1 and 3.

Table 1: Matrix of p-values of individual electronic nose sensor detections of untainted wine (VTQ), synthetically tainted wine (VTQ+STD) and naturally tainted wine (VC) of sensors 1 to 8 after 0, 1 and 2 days from the opening of the wine bottle. Grayed cells refer to p-values larger than 0.05.

Elapsed time (d)	S1	S2	S3	S4	S5	S6	S7	S8
0	0.0172	0.0422	0.0221	0.0070	0.0279	0.0098	0.0203	0.0665
1	0.6427	0.6011	0.7501	0.9524	0.8307	0.8307	0.9912	0.8583
2	0.1729	0.0002	0.0000	0.0082	0.0086	0.0001	0.0001	0.0026

Given that analyses should be carried out on the first day, the question may be raised as to whether the instrumental and operating set-up can be simplified by substituting nitrogen with air. This case was indeed examined but it was found that the separation between tainted and untainted samples is questionable. A source of purified air would possibly restore the feasibility, but would also defeat the simplification sought for.

4. Conclusion

A novel analysis of the Brett aromatic taints by using an electronic nose, a fully recirculating sampling device, and unsupervised classification by bi-dimensional principal component analysis is reported. A mindful choice of the input data showed that the classification map can be identified with reasonable clarity by using promptly analysed samples, while carelessly mixing samples w. Ares with prevalence of untainted wine and tainted wine can be identified, provided that stripping and oxidation effects due to prolonged exposure to air are avoided.

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