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THE USE OF A GC-ELECTRONIC NOSE FOR THE SELECTION OF A WINEMAKING PROTOCOL LEADING TO AN ENHANCED VOLATILE PROFILE IN WINES FROM AROMATIC GRAPE VARIETIES

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Abstract

The multiple oenological materials used in winemaking protocols have various influences on the final aromatic profile of wine. Therefore, their overall effect on a certain variety is difficult to determine, even when some of the materials are tested separately. To simplify the decision, if the winemaker would simply like to enhance the number or the concentration of the compounds forming the volatile profile of a wine, we propose in this paper a method which uses an electronic nose based on flash gas chromatographic technique to test the effect of a certain winemaking protocol. The test was made on two aromatic Romanian autochthonous varieties, Busuioaca de Bohotin and Tamâioasa românească, each vinified with 3 different winemaking protocols. The volatile profiles for each winemaking protocol and variety were recorded and compared by using multivariate statistical analysis, in order to pair the variety and protocol which can generate a more intense volatile profile.

Key words: *electronic nose, volatile profile, aromatic wine, Busuioaca de Bohotin, Tamâioasa românească.*

INTRODUCTION

The aromatic profile of a wine is a trait on which many consumers base their choice. For this reason many researchers have taken into account the possibilities to modify the volatile profile of a wine by using various winemaking protocols.

The main influences on the aromatic profile of the wines are imposed by the grape variety (Rocha et al., 2010). However, even for the same grape variety, the styles of wines possible to obtain vary, in accordance with the substances extracted from the skins by maceration (González-Pombo et al., 2014; Lao et al., 1997) and also by the volatile compounds released by enzymes or yeasts (Palmeri and Spagna, 2007; Cabaroglu et al., 2003) from heavier molecules, called aroma precursors, which are usually glycosides of these aroma compounds. Both advanced extraction and aroma release from precursors are achieved by treatments with specific enzymes (Piñeiro et al., 2006) or presence of specific yeasts (Loscos et al., 2007; Hernandez-Orte et al., 2008).

Other influences on aroma profile of a wine are induced by fermentation, when the secondary aroma of the wine is formed (Sumby et al., 2010). Here too we have several influences. The fermentation aroma is mainly determined by the yeast used for the winemaking process (Ubeda Iranzo et al., 2000; Swiegers and Pretorius, 2005; Swiegers et al., 2009; Samoticha et al., 2017), but raw material itself (Ghaste et al., 2015) and the fermentation activator (Marks et al., 2003; Barbosa et al., 2009; Ugliano et al., 2009) may also induce perceivable differences.

When producing wines, even from the same variety, numerous combinations of enzymes, activators and yeast are possible, all being included in what we call a winemaking protocol, and all having various influences in accordance to the grape variety and performed treatments (Piñeiro et al., 2006).

When applying a winemaking protocol, it is difficult to predict how the several oenological material and treatments entailed in this protocol are going to influence the aroma profile. Even if each oenological material is tested separately, it is not certain that the winemaker is able to predict the final result when combining the materials into a certain protocol.

Thus, to simplify the decision, if the winemaker would simply like to enhance the number or concentration of the components of the volatile profile of a wine, it is possible to test the effect of a certain winemaking protocol by the use of an electronic nose based on flash gas chromatographic technique. In this way, the testing of separate oenological materials which are part of a winemaking protocol is not anymore necessary, this method of evaluating only the final profile saving time and effort.

MATERIALS AND METHODS

Wines of aromatic grape varieties Tamâioasa românească and Busuioaca de Bohotin were produced at industrial scale in volumes of 300 hl. Each variety was vinified by using 3 different protocols (technological schemes), making use of specific enzyme treatments and specific yeast and fermentation activators. As the winemaking protocols are based on commercial products, in order to avoid conflict of interests, the brand names and producers are not disclosed in this paper.

The wine samples prepared are generically described in Table 1.

The volatile profile of each wine variant was determined by a flash gas chromatograph with two short different polarity columns, working on the principle of the electronic nose.

The apparatus from the Alpha-MOS, France, is fitted with a DB-5 (non-polar) and a BD-1701 (slightly polar) 2 m columns and for the chromatographic peak recording two flame ionization detectors, one for each column, thus resulting two simultaneous chromatograms with an acquisition time of 40 s.

Table 1. Protocol description and codification of experimental wines

Wine sample	Experimental protocol					
	Variety	Protocol code	Extraction enzyme	Clarifying enzyme	Fermentation activator	Yeast
BB_AP	Busuioaca de Bohotin	AP	E1	C1	A1	Y1
BB_ED		ED	E2	no	A2	Y2
BB_LA		LA	E3	C3	A3	Y3
TR_AP	Tamâioasa româneasca	AP	E1	C1	A1	Y1
TR_ED		ED	E2	no	A2	Y2
TR_LA		LA	E3	C3	A3	Y3

Each wine sample was injected in the e-nose in triplicate, using the method developed in our laboratory for wines (Antoce and Namolosanu, 2011; Antoce et al., 2015). The main parameters are: injection volume 2500 µl, trap (40°C, pre-purging time 5 s, preheating 20 s, baking 60 s, desorption temperature 250°C), column (heating from 40°C to 200°C with 5°C/s increment, maintaining 2 s the initial and 5 s the final temperature), injection at 200°C, detector temperature 220°C.

The data recording and processing is based on the AlphaSoft version 12.42 and Arochembase library.

Several multivariate statistical analyses are used and compared for data processing, such as the Principal Component Analysis (PCA), which can show the differences in the volatile profiles in accordance to the winemaking protocol used (evaluate discrimination performance), the Discriminant Factorial Analysis (DFA), which can be used to separate in clusters the samples with similar volatile profiles and when necessary classify the unknown samples into these clusters, and finally to determine odor distances from one cluster used as reference by Statistical Quality Control (SQC) analysis.

RESULTS AND DISCUSSION

The electronic nose is able to discriminate the samples in accordance to their raw material – the grape variety – but also to the winemaking protocol used for their preparation, being capable to identify the samples with enhanced volatile profile.

a) Discrimination of grape variety and winemaking protocol by PCA

By applying a Principal Component Analysis a good discrimination (with a discrimination index of 83) can be obtained of the wine sample clusters containing the same variety and winemaking protocol (Figure 1).

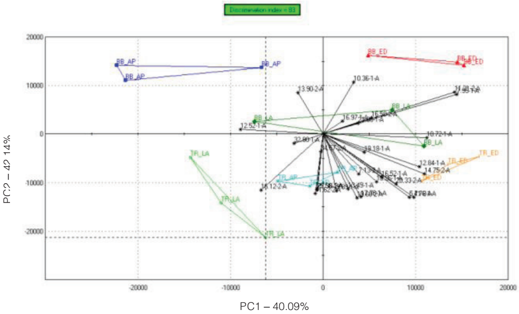

Figure 1. PCA diagram showing the discrimination by the electronic nose of the groups of samples by grape varieties (BB-dark colours and TR-light colours) and winemaking protocols (AP- light/dark blue, ED- light/dark red, LA- light/dark green)

It can be seen that the PC2 axis, which accounts for 42% of the data variability, includes the variables which are dependent on the variety; thus, the clusters of Busuioaca are placed in the upper part of the diagram, while the clusters of Tamaioasa are placed in the lower part of the diagram.

The winemaking protocols are mostly discriminated by the variables included in the PC1, which accounts for 48% of the data variability. Irrespective of the variety, the samples obtained with winemaking protocols AP and LA are placed on the left and those with winemaking protocol ED are placed on the right of the diagram.

This behaviour is most likely determined by the common volatile substances produced by the specific yeast used in each protocol, and not by the specific enzymes used, which also had some influences on the compounds extracted form the grapes or released from grape aromatic precursors.

The third PC only accounts for 4.96% of the variability (Figure 2) and is probably related to the differences induced both by the grape variety and the wine protocol - thus most likely by the type of the enzymes used in each winemaking process.

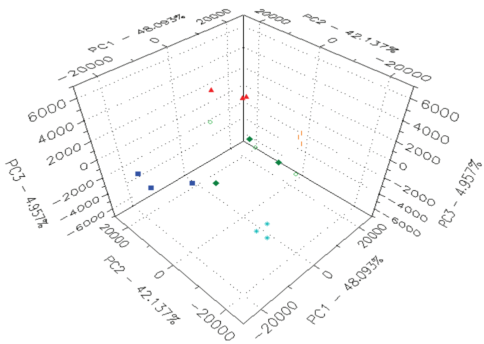

Figure 2. 3D-PCA diagram showing the discrimination by the electronic nose of the groups of samples by grape varieties and winemaking protocols

From Figure 1 we can also see that the most discriminant chromatographic peaks for Busuioaca grape variety are 13.90-2A and 10.36-1A, which were identified as ethyl 2-methyl-butanoate and buthyl acetate, respect-tively, while for Tamaioasa there were more peaks, among which we can cite 25.58-2A (linalool), 11.62-2A (ethyl butanoate), 13.49-1A (isoamyl acetate), 17.76 1A (β-pinene), 19.60-2A (ethyl hexanoate).

Even more peaks contributed to the discrimination of the winemaking protocol, their importance in discrimination being difficult de determine.

b) Discrimination of grape variety and winemaking protocol by DFA

A similar behaviour of the sample clusters as that described in the PCA diagrams can be observed when the Discriminant Factor Analysis is applied (Figure 3). Most of the variation is included in the DF 1 (79.53%), which is related to the grape variety.

The variation determined by the winemaking protocol (the yeast and to a certain degree the enzymes) is included mainly in the DF2 (16.75%) and DF3 (2.74) factors.

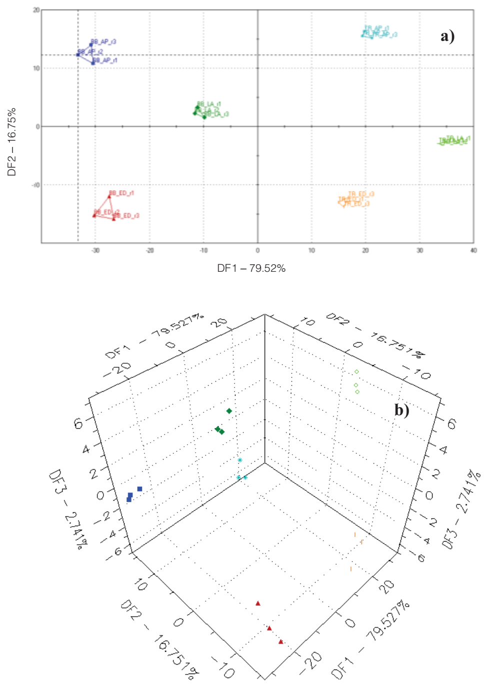

Figure 3. FDA (3a) and 3D-FDA (3b) diagram showing the discrimination by the electronic nose of the groups of samples by grape varieties and winemaking protocols

c) Discrimination of the influences induced by the winemaking protocols for Busuioaca de Bohotin

When only samples obtained based on the same variety are compared, the variability is reduced and the results show only the influence of the winemaking protocol.

The DFA analysis for the Busuioaca de Bohotin allows for discrimination among the samples clusters, but this time clusters are placed much closer to one another (Figure 4).

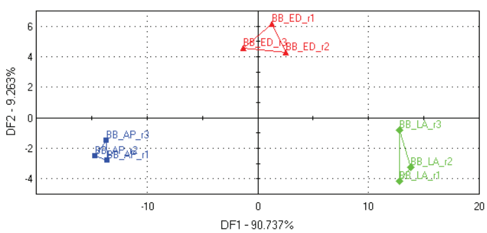

Figure 4. Discrimination by DF Analysis of the groups of Busuioaca de Bohotin samples obtained by different winemaking protocols

The influence of the winemaking protocol for Busuioaca de Bohotin is not much, most of the variability being included in the DF1=90.74%. The variability included in DF2 (9.26%) is mostly related the winemaking protocol ED, which is differentiated by the variables included in this function.

The ED protocol stands out also when it comes to the odor intensity, which was the highest among the winemaking protocols (Figure 5).

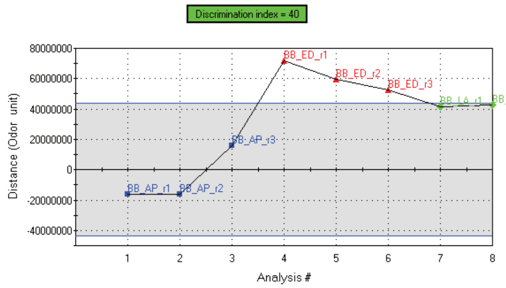

Figure 5. Odor intensity distances of Busuioaca de Bohotin samples obtained by different winemaking protocols

The clusters of BB samples produced with the ED protocol clearly differentiated from the samples produced with protocols AP and LA, when the odor intensity assessed by measuring the odor distances among the samples were determined.

This higher concentration of volatile substances in the wine profile could be a good indication of fermentation with a yeast more effective in producing secondary metabolites, but this does not necessarily indicate a better aromatic profile. On the contrary, the wines produced with the ED protocol ranked last among the three protocols on a sensory evaluation based on the OIV score sheet: BB-ED = 84, BB-LA = 88 and BB-AP = 90 points.

d) Discrimination of the influences induced by the winemaking protocols for Tamâioasa romaneasca

In the case of Tamaioasa romaneasca the winemaking protocol had a higher influence than in the case of Busuioaca de Bohotin (Figure 6). The variability induced by the winemaking protocol was 83.22 included on DF1 and 16.78% in DF2, the last one being mostly related to the LA protocol.

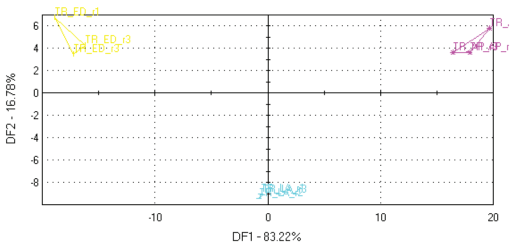

Figure 6. Discrimination by DF Analysis of the groups of Tamaioasa romaneasca samples obtained by different winemaking protocols

For this variety, the highest odor intensities were also displayed by the wines based on ED protocol (Figure 7), confirming that this protocol is clearly different and identifiable by the E-nose, irrespective of the raw material used.

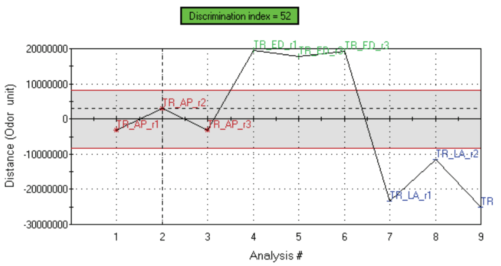

Figure 7. Odor intensity distances among the groups of Tamaioasa romaneasca samples obtained by different winemaking protocols

The sensory evaluation based on the OIV score sheet showed the following ranking: TR-ED and TR-LA = 86 points and TR-AP = 92 points. This shows that the high intensity volatile profile was not preferred by the winetasters, but neither was the lowest intensity profile (LA protocol).

CONCLUSIONS

Based on the evaluation of the volatile profiles of the wines produced from the two aromatic varieties using three different winemaking protocols, the following conclusions can be drawn:

- The variety is most likely discriminated by the electronic nose based on the terpenic volatile profile of each variety, but also by other esters and acetates either from the primary or secondary aroma.
- The winemaking protocol has two main components, with different influences: the enzyme influences the primary aroma by changing the level of extraction of several aromatic compounds and also the degree of release of the aromatic compounds from precursors, while the yeast influences the secondary/fermentation aroma, by the esters and acetates that it forms during fermentation. The yeast has the main influence in the aromatic profile of wines obtained from the same variety.
- Irrespective of the grape variety used, the ED winemaking protocol stands out from the three different protocols, with larger distances measured in odor intensity units, compared to the other two protocols. A higher concentration of the volatile substances measured for this winemaking protocol may indicate a higher aroma intensity which can be perceived sensorially, but it may not necessary mean that the consumers will prefer the resulted wines. It is however a good indication that the wines will be more intense in the nose, a trait that some consumers like.
- For the final protocol selection the results of e-nose testing should always be correlated with the sensory analysis results.
- This type of e-nose analysis may be particularly useful when more than three winemaking protocols are under evaluation and preparation of many wines on industrial scale may be not an economical option.

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