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THE INFLUENCE OF CHITOSAN TREATMENTS IN ORGANIC FETEASCA NEAGRA VINEYARD ON THE AROMATIC PROFILE OF WINES EVALUATED BY ELECTRONIC NOSE

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Abstract

The present study evaluates the differences in the aroma profile of wines, induced by the chitosan treatments of Feteasca neagra grapes organically cultivated. The treatments of the grapes included a variant only with Bordeaux mixture (5 kg/ha), as a control, a variant only with chitosan (5 kg/ha), and another variant treated with both chitosan and Bordeaux mixture (5+5 kg/ha). The aroma of the resulted wines produced by the classical maceration-fermentation technology was analysed by an electronic nose working on the principle of fast GC. Several organic volatile compounds with impact on aroma were identified and their relative quantities compared for each type of treatment. Esters (fruity scents) are the main aroma compounds found, but some other compounds more related to vegetal aroma were also present. The electronic nose clearly identified each type of wine in accordance to the treatment in the vineyard. The principal component analysis separated the wines based on their floral-fruity-vegetal notes (PC1) versus grassy notes (PC2). SIMCA analysis showed that, compared to the control samples with Bordeaux mixture, the samples from grapes treated with chitosan or chitosan and Bordeaux mixture placed closer in the space of odour and outside of the space of control wines. Thus, this preliminary study showed that chitosan treatment in vineyard induces measurable olfactory differences in the wines.

Key words: *chitosan vine treatment, electronic nose, e-nose, Feteasca neagra, wine aroma profile.*

INTRODUCTION

Chitosan is a natural compound extracted form insects, which is used for plant protection (Hadrami et al., 2010) and is also allowed in organic agriculture. Chitosan hydrochloride was found to enhance plant protection against pathogenic bacterial or fungi infections, such as *Plasmopara viticola* (Dagostin, 2006.)

It can be an alternative to the treatments based on copper that are used with high frequency in organic viticulture, which are very effective but, in the long run, lead to copper accumulation in the soil and harm the vine itself due to its phytotoxicity (Toselli et al., 2009).

Chitosan treatments can replace or, at least can contribute to the reduction of copper usage.

In the vineyard chitosan may be used for treatments based on its several documented insecticidal (Badawy and El-Aswad (2012) and fungicidal effect (Garde-Cerdán et al., 2017; Matei et al., 2019), but so far consistent treatment plans have not been established. The effect grape treatment with chitosan has on the wine produced afterwards from these grapes was not sufficiently investigated, therefore, this type of research should be conducted to determine several aspects related to wine quality parameters. In order to achieve the stated objective, in the present study, the samples obtained with various interventions in the technology of ecological culture were evaluated in order to determine the profile of volatile substances and to identify the differences induced by the treatments. The samples were tested using an electronic nose analyzer, based on flash chromatography, by taking volatile substances from the sample by the "headspace" method (i.e. from the gaseous part above the sample in a chromatography vial). The "headspace" method allows the analysis of the compounds present in the gas phase, supposedly those compounds that the human nose would perceive when a wine sample is smelled.

MATERIALS AND METHODS

Grapes of Feteasca neagra were cultivated at the Research Station for Viticulture and Oenology Murfatlar under 3 various types of treatments in the vineyard. More data about the vine plantation can be found in previous publications (Artem et al., 2021ab; Artem et al, 2020).

The experimental variants, with the vine treatments, product quantities and number of treatments are described in Table 1. Chitosan was procured from Kitozyme SA, Belgia and the Bordeaux Mixture from SC Verdon Solution SRL, Romania.

Table 1. Treatments used for Feteasca neagra grapes used for the experimental wine variants

Variant code of wine	Type of vine treatment		Number of treatments
	Bordeaux mixture	Chitosan	
FN20-Bord (control)	5 kg/ha	0 kg/ha	12
FN20-Chit	0 kg/ha	5 kg/ha	12
FN20-ChiBo	5 kg/ha	5 kg/ha	6

The wines were produced in the autumn of 2020 from grapes of Feteasca neagra organically grown. The volatile substance profiles of the experimental wines were determined using a Heracles electronic nose (Alpha MOS, France), which works on the principle of flash gas chromatography, using two short columns with different polarities (one non-polar DB5 - 5% diphenyl, 95% dimethylpolysiloxan and one medium-low polar DB1701 - 14% cyano-propylphenyl, 86% dimethylpolysiloxan). The two columns have flame ionization detectors (FID) located at the end, providing two chromatograms that are recorded simultaneously. More details regarding the apparatus and the method used for recording and analysing the volatile profile can be found in previously published papers (Antoce and Cojocaru, 2021; Cojocaru and Antoce, 2019; Antoce and Namolosanu, 2011).

The software AlphaSoft v12.42 is used to control the chromatograph and to record and process data. Various statistical methods are available, the ones selected for this study being principal component analysis (PCA), discriminant function analysis (DFA) and SIMCA.

Some of the volatile substances which are recorded as chromatographic peaks can be identified using an integrated database of chemical compounds, AroChemBase 2010.

RESULTS AND DISCUSSIONS

1. Main volatile substances identified by the e-nose in Feteasca neagra wines

The main volatile substances were identified by the electronic nose as part of the aromatic profile of Fetească neagră wines obtained with various interventions in organic farming technology by using the AroChemBase database that the device is equipped with. Relevant volatile compounds of experimental wines are identified and presented in detail in Table 2, along with their main sensory attributes. The sensory descriptors associated with a particular volatile organic compound are based on information provided by AroChemBase and other databases, such as ChemSpider.

Table 2. Relevant volatile organic compounds identified in wine samples from Feteasca neagră obtained from ecologically treated plots with different treatments

Column DB5				Column DB1701			
Average retention time (RT)*	Kovats Indices/ Sensors	Identified compound	Sensory descriptors	Average retention time (RT)*	Kovats Indices/ Sensors	Identified compound	Sensory descriptors
10.43	810.80-1	ethyl lactate	fruits	10.04	820.36-2	3-mercapto-2-butanone	sulfurous, onion
10.76	819.10-1	3-hydroxy-2-pentanone	grass, truffle	14.83	941.16-2	3-methylbutyl acetate	banana, pear
12.93	873.53-1	3-methylbutyl acetate	banana, pear	16.52	981.96-2	1-hexane-ol	sweet, woody, green, herbaceous
17.77	992.23-1	ethyl hexanoate	apples, bananas, wine, pineapples	19.60	1,058.08-2	ethyl hexanoate	apples, bananas, wine, pineapples
22.40	1,108.53-1	2-phenylethanol	floral, honey, rose	28.06	1,278.58-2	2-phenylethanol	floral, honey, rose
32.82	1,387.40-1	ethyl decanoate	grapes, pears, oily, sweet, waxy, fruity, apple, brandy, soapy	34.57	1,456.03-2	ethyl decanoate	grapes, pears, oily, sweet, waxy, fruity, apple, brandy, soapy

*average values of 3 recorded chromatograms (repetitions of the same sample); **the sample code consists of the Kovats index and the column on which the chromatogram was recorded (1 = DB5; 2 = DB1701); ***Sensory descriptions for identified compounds are retrieved from AroChemBase and other public databases.

The volatile compounds identified in the aromatic profile of these red wines showed different concentrations in samples from grapes from ecologically treated plots with different treatments, which leads to a different overall flavour for each group of wines. In order to be able to highlight the differences in concentration, the peak areas corresponding to each compound identified in the samples with different treatments were compared (Table 3).

By applying the analysis of variance (ANOVA) and the Tukey test to compare the means in pairs, it was possible to determine the main significant differences that appear between the experimental variants. Thus, in the sample treated with Bordeaux mixture, FN20_Bord, the presence of significantly increased concentrations of esters such as ethyl lactate, ethyl hexanoate and ethyl decanoate is noted, which each of them bringing, and especially in combination, a fruity note (apples, bananas, pineapple, grapes). The FN20_ChiBo sample specifically shows more vegetal notes, due to a higher content of 1-hexanol (raw, green note) and 3-mercapto-2-butanone (onion note). These special aromatic notes are appreciated in some wines, especially in red ones, which makes this sample stand out more easily than other experimental wines.

Table 3. Chromatographic peak surface area of relevant volatile organic compounds identified in Feteasca neagră wine samples obtained from ecologically treated plots with different treatments

	FN20_Bord	FN20_ChiBo	FN20_Chit
Column DB5			
ethyl lactate	**1238±102[a]**	810±155[b]	1123±228[a]
3-hydroxy-2-pentanone	1982±172[a]	2134±292[a]	1723±253[a]
3-methylbutyl acetate	38059±1540[a]	37308±2057[a]	40835±3363[a]
ethyl hexanoate	**13574±406[a]**	11487±688[b]	11071±1341[b]
2-phenylethanol	3754±530[a]	3048±591[b]	4315±676[a]
ethyl decanoate	10973±631[a]	9919±655[a]	10658±759[a]
Column DB1701			
3-mercapto-2-butanone	2078±66[b]	**2312±140[a]**	2036±145[b]
3-methylbutyl acetate	27262±893[b]	27385±1123[ab]	29786±2135[a]
1-hexane-ol	636±29[b]	**727±42[a]**	618±16[b]
ethyl hexanoate	**9791±221[a]**	8241±426[b]	7984±887[b]
2-phenylethanol	2487±341[ab]	2118±145[b]	2900±336[a]
ethyl decanoate	**8117±78[a]**	7135±379[b]	7862±448[a]

*The different letters show that there is a significant difference between those samples at a probability level of 95% (α = 0.05). The statistical analyses applied were the ANOVA and Tukey test. The averages with the highest value, if significantly different from those in other samples, are marked bold.

2. Electronic nose discrimination of Fetească neagră wine groups obtained with various interventions in organic farming technology

Another attempt to analyse the data provided by the electronic nose focused on the possibility of discriminating wine samples produced from plots treated ecologically with different substances.

The substances included in Tables 2 and 3 are all that can be identified using the electronic nose database for GC and may be present in different amounts and combinations in the experimental wine samples analysed. However, the Heracles analyser does not quantify these substances, the discrimination of the samples being made only on the basis of differences in their specific combinations of volatile organic compounds (fingerprints of each wine). This is one of the advantages of using this e-nose technology, namely that conclusions can be drawn in a faster and cheaper way, without having to invest in GC with mass spectroscopy to quantify the identified compounds. Thus, these chromategraphic substances / peaks were identified only to determine if they are more correlated than others with a certain type of treatment performed in the vineyard.

However, the correlation of a certain treatment with several volatile substances in wine is easier to observe in the PCA and DFA graphs, especially in the bi-plot type, which separates the sample groups, but also presents the compounds (vectors) with the highest probability of inducing separation.

The PCA analysis shows that the experimental ecological samples of Fetească neagră obtained with various interventions in culture technology can be differentiated with the help of the electronic nose based on the profile of volatile substances with discrimination power over 0.5, the discrimination index being positive (Figure 1). Although the value of the discrimination index is not very high, there is a clear differentiation of the groups of samples, especially the samples resulting from plots treated with Bordeaux mixture (FN20_Bord), compared to the other two groups, which had treatments that included chitosan (FN20_ChiBo and FN20_Chit), which are closer to each other in the two-dimensional PCA space.

The two main components comprise 97.41% of the total variance, with PC1 including 67.08% of the variance and PC2 30.33%.

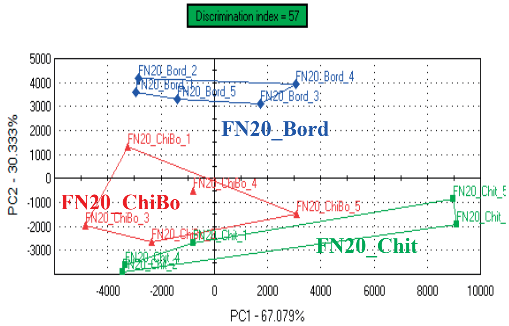

Figure 1. Analysis of the Principal Components (PC1 and PC2) for the experimental ecological wines of Fetească neagră obtained with various interventions in the culture technology (discriminative sensors with power greater than 55%)

In general, it is sufficient to include variations induced by the presence of various volatile substances in wine samples in only two main components, but sometimes, when the separation is not clear enough, a third component can be used, resulting in a three-dimensional space (Figure 2).

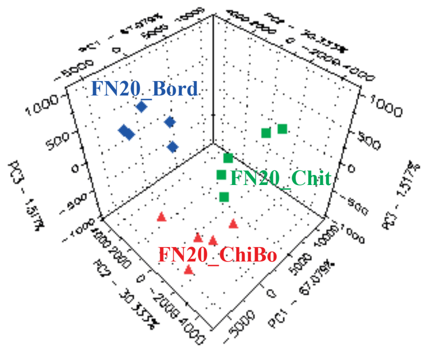

Figure 2. Separation by PCA-3D analysis of the experimental wine samples of Fetească neagră according to 3 main components (PC1, PC2 and PC3)

In the three-dimensional PCA space, the separation is clearer, noting that each wine group has an independent olfactory/aromatic identity. The 3 main components explain 98.92% of the variation of aromatic profiles (PC1 67.08%, PC2 30.33% and PC3 1.51%). However, even this separation shows that chitosan treatments lead to sensory profiles of wines closer to each other compared to the sensory profile of control wine (FN20_Bord).

PCA analysis performed with a selection of sensors represented by chromatographic peaks whose substances have been clearly identified, compared to those based on the selection of all sensors with over 55% discrimination power, lead to a better discrimination of wines produced with technologies different vineyards. Thus, in Figure 3 it is observed that the volatile substances identified by the electronic nose and reported in Tables 2 and 3 lead to the explanation of the total variability in proportion of 95.25% (PC1 = 75.29%, PC2 = 19.96%), having a higher discrimination index (81 when taking into account the identified substances, compared to 57 when taking into account all electronic nose sensors with more than 55% discriminating power).

The principal component PC1 mainly includes substances that induce pleasant aromas in wines: 3-methylbutyl acetate (banana, pear), 2-phenylethanol (floral, honey, rose), 3-mercapto-2-butanone (vegetable), 1-hexane-ol (sweet, woody, green), while the PC2 component predominantly includes shades of raw and green such as ethyl hexanoate (green, herbaceous) and 3-hydroxy-2-pentanone (grass). Ethyl decanoate (grapes, fruity) and ethyl lactate (fruity) are equally present in both principal components. We can thus consider the PC1 axis as the floral-fruity-vegetable axis, and the PC2 axis as the one with a grassy aroma. The PC1 axis identifies two different trends: a floral-fruity area for the left quadrants of the graph and a vegetable-woody area for the right quadrants of the graph.

The sample group that includes the Bordeaux mixture treatment (control, FN20_Bord) is mainly influenced by the herbaceous component (PC2). Samples from the chitosan treatment group (FN20_Chit) are majorly influenced by the floral-fruity component of the PC1 axis. The samples in the group treatment of Bordeaux mixture of chitosan juice and chitosan (FN20_ChiBo) are rather correlated with the compounds on the PC1 axis that create vegetable flavours.

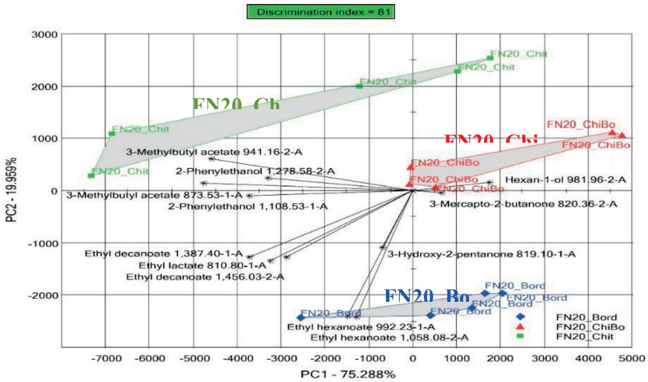

Figure 3. Bi-plot PCA for the experimental ecological wines of Fetească neagră obtained with various interventions in the culture technology

DFA analyses were also performed for the 3 groups of samples, and the results are included in Figures 4 and 5. Thus, the analysis of the discriminant factors was applied to the values of the relative area (RA) of certain chromatographic peaks (e-nose sensors), selected by the procedure described above.

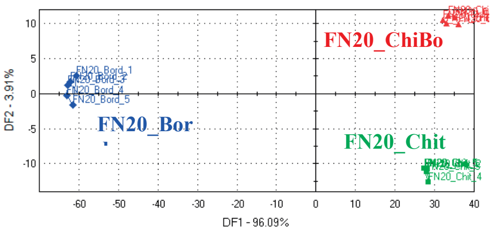

Figure 4. Analysis of Discriminatory Factors (DF1 and DF2) for discriminating groups of experimental organic wines of Fetească neagră obtained with various interventions in culture technology (discriminatory sensors with power greater than 55%)

As we can see in Figures 4 and 5, the groups of samples analyzed (FN20_Bord, FN20_Chit, FN20_ChiBo) are well discriminated and separated on the DFA graph, based on the differences in concentration of the compounds that define all discriminant sensors with power greater than 55%. (Figure 4), as well as based only on the identified volatile compounds included in Tables 2 and 3 (Figure 5).

The general DFA analysis (Figure 4) shows that the first two dimensions explained in full (100%) the total variance observed for our samples, with 96.09% and 3.91% of the variance of the data explained by DF1 and DF2, respectively.

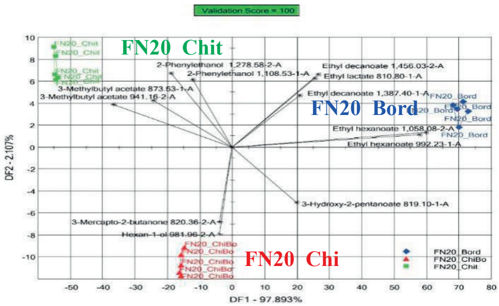

Figure 5. DFA bi-plot for the experimental ecological wines of Fetească neagră obtained with various interventions in the culture technology

The DFA bi-plot, which places the sample groups in the field containing the volatile substances identified in their sensory profile, also shows that the first two dimensions explained in full (100%) the observed variance for our samples, with DF1 = 97.89% and DF2 = 2.11%. This DFA chart also shows that wine groups tend to have different dominant flavours. Thus, it is confirmed that the control FN20_Bord is defined by the ester aromas of ethyl hexanoate (green, herbaceous) combined with those of ethyl decanoate (grapes, fruit), FN20_Chit samples made from chitosan-treated plots are more floral-fruity with flavours of 3-methylbutyl acetate (banana, pear) and 2-phenylethanol (floral, honey, rose), and those of FN20_ChiBo treated with both substances have a predominant vegetal scent due to 3-mercapto-2-butanone (vegetable), 1-hexane-ol (sweet, woody, green).

In Figure 6, the SIMCA diagram also shows that compared to the control with Bordeaux mixture, the samples with treatments containing chitosan are placed outside the olfactory space of the control, and are very close to each other.

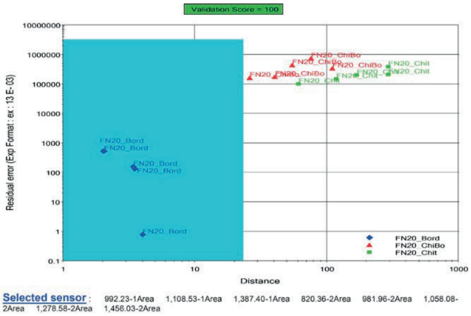

Figure 6. SIMCA diagram for determining the olfactory differences between the groups of organic Fetească neagră wines obtained with various interventions in the culture technology

CONCLUSIONS

Electronic nose determinations indicate that samples of chitosan-treated grapes showed volatile profiles of wines closer to each other compared to the volatile profile of control wine (FN20_Bord).

Control FN20_Bord is defined by the ester aromas of ethyl hexanoate (green, herbaceous) combined with those of ethyl decanoate (grapes, fruit); FN20_Chit samples, made from chitosan-treated plots, are more floral-fruity with aromas of 3-methylbutyl acetate (banana, pear) and 2-phenylethanol (floral, honey, rose); and those of FN20_ChiBo treated with both substances have a dominant vegetable due to 3-mercapto-2-butanone (vegetable) and 1-hexane-ol (sweet, woody, green).

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