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CHITOSAN TREATMENTS IN ORGANIC VINEYARD AND THEIR IMPACT ON THE COLOUR AND SENSORY PARAMETERS OF FETEASCA NEAGRA WINES

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Abstract

Chitosan is a natural polymer approved for the treatments of organic vineyards based on its fungicide effect. Beside the protection it offers, chitosan has also the potential to modulate polyphenolic content in the black grapes, hence improving the colour of the resulted red wines. The present study on Feteasca neagra variety organically cultivated showed that there was indeed an improvement of the total polyphenolic content and colour of wines obtained from grapes treated with chitosan (5 kg/ha), as compared to the wines from grapes only subjected to the usual treatment based on Bordeaux mixture (5 kg/ha). The study included a mixed treatment, with both chitosan and Bordeaux mixture (5+5 kg/ha). The increase of total polyphenols and colour is apparent in all samples treated with chitosan, being higher when chitosan was used alone than in the case of the mixed treatment. The sensory qualities of the wines were also influenced. While the main sensory parameters of the wines were not significantly affected by the vineyard treatments, the aromatic profiles perceived sensorially showed that the floral scent decreased and the spiciness increased due to chitosan treatment, the effect being more evident in the case of chitosan used alone. These preliminary results suggest that chitosan can be useful for the modulation of the wine quality and style.

***Key words**: chitosan vine treatment, wine colour parameters, Feteasca neagra, wine sensory quality.*

INTRODUCTION

Chitosan (poly-D-glucosamine, CAS No. 9012-76-4) is a deacetylated derivative of chitin, being a biopolymer present in the exoskeleton of insects and in crustacean shells, but also in some microorganisms, especially in the fungal cell wall (Ma et al., 2022; Apetroaei et al., 2016).
Because it is a non-allergenic product, with low toxicity, chitosan has multiple uses, as a phytosanitary agent (Iriti et al., 2011), for producing, preserving (Lo'ay and El-Khateeb 2018) or packing food (Oladzadabbasabadi et al., 2022), for filtering and purifying water, in certain products for medical use such as in bandages for wound healing (Kim, 2010).
Being natural and biodegradable, it is authorized for use in organic agricultural treatments, but also for the treatment of (organic) wine, especially because it has a high positive charge at the wine pH and has the physico-chemical property of flocculating, thus being a stabilizing agent.
In plants it is an antifungal protector, but also an elicitor of plant growth (Gutiérrez-Gamboa et al., 2019). Chitosan increases the plant's immune response by producing antioxidant enzymes, more polyphenols (Vitalini et al., 2011), as a result of increased expression of certain genes, etc. For plants, chitosan is also a modulator of water loss (Bittelli et al., 2011). Under conditions of water stress, chitosan reduces perspiration, closing even the stomata, but when there is too much water it can increase the perspiration rate by about 30%.
Chitosan also increases plant firmness (Adamuchio-Oliveira et al., 2020). In response to chitosan, certain phytoalexins such as chitinase and lignin are produced. This is because the plant recognizes chitosan as a component (or derivative) of insects and fungi, so it responds by increasing those substances that can prevent the attack of insects and fungi,

such as increased lignin levels, which makes the plants harder to be penetrated. Because pathogenic fungi have more chitin than others, chitosan treatments affect more these fungi and not the useful ones such as mycorrhizal microorganisms.

In this way chitosan can be seriously considered as an alternative to copper treatment in organic and conventional vineyards (Dagostin et al., 2006a, 2006b, 2011).

In this study, in order to improve existing organic farming technologies, chitosan treatments, alone or in combination with Bordeaux mixture, were carried out in the vineyard during 2020 in order to determine whether this new material leads to better grapes and, respectively, higher wine quality. Hereafter, the effect of chitosan vine treatments on the wines produced from organic grapes is assessed, with special focus on colour, polyphenol composition and sensory characteristics.

MATERIALS AND METHODS

An organic Feteasca neagra vineyard cultivated in Murfatlar (Artem et al., 2021a, 2012b, Artem et al., 2020) was used to compare the effect of chitosan treatments with the usual application of Bordeaux mixture.

The phytosanitary protection treatments applied consisted of doses of 5 kg/ha of each substance (chitosan or Bordeaux mixture), applied alone or in combination. Applied together, the two substances were in a dose of 5 kg/ha each, but the sprayings were applied less often, i.e. 6 treatments/year instead of 12, as it was done with the substances used alone.

The wines which resulted in 2020 from the processing of these grapes differently treated are named in correlation with the type of treatment in the vineyard: FN20-Bord, FN20-Chit and FN20-ChiBo, respectively. The variant treated only with Bordeaux juice is considered a control, because this is the classical treatment applied in organic viticulture.

The physico-chemical parameters of wines were measured, to establish the differences between the treatments, but also to evaluate the intrinsic quality of the wines and their compliance with the legislation. The laboratory methods recognized by the OIV (International Vine and Wine Organization, 2021) were used.

To describe the sensory quality of wines and determine the samples with the highest commercial chances, the most used methods are sensory profile analyses, based on specially designed evaluation sheets. Such a score sheet was developed at USAMV and is part of a patent (Antoce and Namolosanu, 2007). With this sheet, the following elements were evaluated:

- general sensory characteristics of wines (acidity, sweet taste, extract, colour intensity and aroma intensity), which is done through the usual tasting techniques, using continuous scales for evaluating the intensity of perception, with maximum values of 10;
- specific sensory wine parameters (parameters that are considered essential for defining typicality and quality, such as colour and flavour). For the evaluation of the specific parameters, scoring scales with discrete values are used, represented in the form of 5 boxes, which can be considered as notes with integer values between 1 and 5. To use them in mathematical models together with the grades obtained on the 10-point scales, these values are multiplied by a factor of 2. Thus, the resulting values are all in the range 0-10. For each score given to a specific flavour parameter, a more detailed description of the flavour is required from tasters. For example, for aromatic fruit notes, where they can be identified, the types of fruit can be detailed, more generally such as notes of citrus, berries, tropical fruits, temperate fruits or even more specifically, identifying exactly the fruit from the respective classes, such as orange, grapefruit, raspberry, currant, plum, etc.

Since sensory analysis with human evaluators cannot accurately determine colour shades and small differences in colour intensity, in order to accurately determine whether there are differences between experimental variants induced by vine treatments, the defined colour parameters were measured by the CIEL*ab* method with the help of a spectrophotometer.

The colour was determined by a Jena AG Specord 250 UV-VIS Analytik spectrophotometer, which runs software version WinAspect 2.2.7 for spectrum recording and data storage, and Chroma software, specially designed for colour analysis. The measurements were performed by measuring the transmittance of the wine every 1 nm on the visible spectrum from 400 nm to 700 nm, with a quartz cuvette with a 1 mm optical path and using as standard D65 light and an observation angle of 10°. The software automatically calculates where the samples place in a uniform colour perception space with three dimensions and the associated CIEL*ab* parameters: L, *a, b*. L represents "brightness" or "luminance" - the higher the value, the more "transparent" the sample. According to the CIEL*ab* system, the parameters obtained are:

- coordinate *a*, which shows the placement of the colour between red and green; if *a*> 0 is red, and if *a* <0 is green;
- coordinate *b*, which shows the placement of the colour between yellow and blue; if *b*> 0 is yellow, and if a <0 is blue;
- the parameter L, representing the brightness of a coloured object judged in relation to the brightness that appears as white; more simply is the degree of transparency or opacity (0 represents opacity, 100 complete transparency);
- the parameter c_{ab} or *c* (chrome), representing the chromaticity of a coloured object judged in relation to white, or more simply, the purity, the saturation or the depth of the colour;
- the parameter h_{ab} or h (angle in radians) representing that descriptor of the appearance by which a colour is identified according to its resemblance to the colour red (0°), yellow (90°), green (180°), blue (270°), or a combination of two of these. Simply put, h is the colour shade.

The variations of the parameters (ΔL, Δc_{ab} and Δh_{ab}) can also be calculated, as well as the total colour difference, ΔE_{ab}, in relation to a reference point (control sample), being expressed in CIEL*ab* units. The formula for calculating $\Delta E_{ab} = ((L_c-L_s)^2 + (a_c-a_s)^2 + (b_c-b_s)^2)^{1/2}$, where c=control and s=sample, provides a value that represents the colour difference.

For analysis, 4 ml of each wine sample was centrifuged for 10 minutes at 4000 rpm and then subjected to spectrophotometric determination.

Measurements were performed in 5 repetitions for which the means and standard deviations were calculated. Analysis of variance (ANOVA) was applied for each parameter and, where significant differences were found, the Tukey test was applied for comparison in pairs.

RESULTS AND DISCUSSIONS

1. Evaluation of the main parameters of wines

The quality of wines obtained with various interventions in the technology of ecological culture was firstly evaluated through the physico-chemical analysis. Table 1 shows the results for the main physico-chemical parameters of the wines produced from grapes obtained from experimental plots treated ecologically with Bordeaux mixture, chitosan or a mixture of the two.

Table 1. The main physico-chemical parameters of the wines resulting from grapes obtained from the experimental plots treated ecologically with various technological interventions

Physico-chemical parameters*	FN20-Bord	FN20-Chit	FN20-ChiBo
Alcohol (%)	15.42±0.05[a]	15.92±0.08[b]	15.78±0.12[b]
Potential alcohol (%)	15.54±0.07[a]	**16.13±1.62[a]**	**16.18±0.13[a]**
Reducing sugars (g/l)	2.10±0.25[a]	3.60±0.34[b]	**6.80±0.22[c]**
Total acidity (g/l tartaric acid)	**4.14±0.25[a]**	3.72±0.12[b]	3.50±0.13[b]
Volatile acidity (g/l acetic acid)	0.39±0.03[a]	0.33±0.03[b]	0.34±0.02[b]
pH	3.7±0.1[a]	3.8±0.1[a]	3.8±0.2[a]
Total extract (g/l)	29.5±0.5[a]	33.0±0.6[b]	**35.8±0.4[b]**
Dry extract (g/l)	27.4±0.1[a]	**29.1±0.4[b]**	**29.0±0.3[b]**
Total polyphenols (g/l)	1.46±0[a]	**1.65±0.3[b]**	**1.60±0.2[b]**

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

It is noted that the chitosan treatments lead to a higher concentration of sugars in the grapes, so that the alcohol content is slightly higher in those samples. Taking into account both the residual sugars and alcoholic concentration obtained, we find that the samples have a potential alcoholic strength of 15.54% for FN20-Board (control), and significantly higher in FN20-Chit (16.13%) and FN20-ChiBo (16.18%).

Also, chitosan leads to an increase in dry extract, as well as in the concentration of total polyphenols, the higher values of both parameters being directly correlated with the quality of the wine.

The treatment with Bordeaux mixture is correlated with higher values of total acidity.

2. *Sensory profile of wines*

The evaluation of the quality of wine included the application of the sensory profile analysis, performed with a panel of trained tasters.

The sensory analysis of the general parameters showed only small differences between the samples of organic Fetească neagră grapes sprayed with different substances (Bordeaux mixture, chitosan or a combination of them). Table 2 shows the averages of the scores given by the tasters to evaluate the perception of the main parameters of the wines.

Table 2. Perception of the main general parameters of the wines evaluated by sensory analysis (notes on a scale from 0 to 10, average values ± standard error)

General parameter*	FN20-Bord	FN20-Chit	FN20-ChiBo
Acidity	4.80 ± 1.21^a	4.53 ± 0.90^a	3.47 ± 2.74^a
Sweetness	0.70 ± 0.35^a	0.50 ± 0.00^a	**2.03 ± 0.81^b**
Astringency	4.90 ± 1.15^a	4.13 ± 1.89^a	5.00 ± 2.50^a
Bitterness	1.43 ± 1.21^a	2.50 ± 0.00^a	2.38 ± 0.53^a
Extract	4.57 ± 0.84^a	4.10 ± 1.39^a	4.97 ± 0.06^a
Colour intensity	6.30 ± 1.11^a	6.07 ± 0.90^a	6.83 ± 1.11^a
Aroma intensity	5.53 ± 1.70^a	4.00 ± 0.87^a	5.07 ± 1.10^a

*Different letters show that there is a significant difference at a probability level of 95% ($\alpha = 0.05$) determined by ANOVA and Tukey test. The averages with the highest values, if significantly different from those in other samples, are marked in bold.

Sensorially, the only significantly different main parameter was the sweetness for FN20-ChiBo sample. The sample was clearly perceived as sweeter, fact confirmed by the chemical analysis (Table 1), which shows that this is a semi-dry wine, unlike the other two, which are dry.

From the sensory analysis of the parameters related to the aroma of the wines, however, it was possible to obtain interesting differences between the wines resulting from the application of different viticultural technologies. Tasters were able to identify certain flavour descriptors, which were summarized in Table 3.

Also, the wines were separately analysed using the score sheet designed for evaluation in wine competitions proposed by the International Organization of Vine and Wine (OIV, 2021) and the scores obtained were also included in Table 3, in order to be correlated with the determined flavour attributes.

The samples were also compared with each other, the tasters determining by consensus the sample with the best olfactory intensity (FN20_Bord), the sample with the lowest sensation of structure (FN20_Chit) and, respectively, the most commercial sample, which could be given in consumption without requiring maturation (FN20_ChiBo).

Table 3. The main descriptors of the wines evaluated by sensory analysis and the scores obtained in the evaluation on a scale of 100 points (OIV score sheet).

	FN20-Bord	FN20-Chit	FN20-ChiBo
Sensory flavour attributes	Bitter cherries	Bitter cherries; smoked	Bitter cherries, sweet cherries, blueberries
Evaluation score (out of 100 points)	82.00	80.67	84.33
Wine description	lively nose, slightly vanilla, good aromatic intensity, but thin	well-structured wine, slightly flat, relatively simple, aromatic, with a high alcohol content, light, drinkable	slightly reductive but fruity, high concentration of alcohol that strengthens the sweet note of residual sugars, very drinkable, with soft tannins, velvety, supple, elegant
Remarks	The sample with the best olfactory intensity	Sample with the slightest sensation of structure	The most commercial sample (can be consumed without passing through a maturation period)

By sensory analysis of specific aroma parameters, the categories of dominant aromas in these wines could be identified, namely: aromatic hints of flowers, fruits, vegetable, complex aromas and spices / toast.

The averages of the values obtained from the tasting panel on the discontinuous scales from 1 to 5 were used to obtain a suggestive diagram, which shows the aromatic imprint (sensory profile of the aromas) of the experimental wine samples (Figure 1).

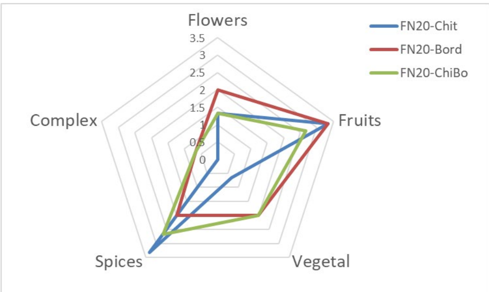

Figure 1. Aromatic profile of Feteasca neagră wines obtained with various interventions in organic cultivation technology

As it can be seen in Figure 1, the wines are relatively simple in aroma, but certain differences can be identified, depending on the treatment carried out in the vineyard. Thus, the chitosan treatments bring a note of burnt, slightly spicy, the Bordeaux mixture creates a greater floral sensation, while the combination Bordeaux mixture-chitosan leads to a wine with a moderate sensory profile, which may be better appreciated by consumers and can be marketed without going through a period of maturation to reduce the note of astringency and the bitterness that young red wines generally have.

In order to better observe the sensory differences between the samples and, especially to determine the parameters with the greatest influence on the discrimination of the samples, the analysis of the principal components was performed, both for the general parameters (Figure 2a) and for specific aroma (Figure 2b).

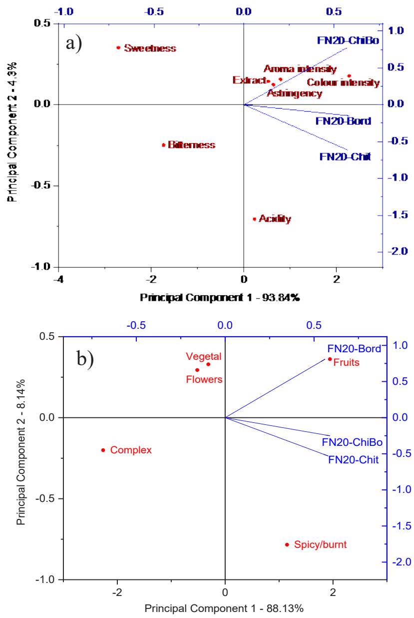

Figure 2. Principal Components Analysis (PCA) for the wines evaluated by sensory analysis: a) main parameters; b) flavour categories

Figure 2a shows that the main physico-chemical parameters, evaluated by tasting, are not substantially influenced by the differences in treatments performed in the vineyard. However, it is noticeable a clearer positioning of the FN20-ChiBo sample in the quadrant determined by the sensory attributes that give quality to the wine, namely "aroma intensity", "colour intensity", "extract", "astringency", which suggests that the combined chitosan-Bordeaux mixture treatment can lead to wines of a higher quality compared to other treatments, in which the substances were used independently. The main components PC1 and PC2 cover a large part of the data variance, of 98.14%, i.e. the evaluated sensory attributes are those that intervene in the creation of the sensory profile of the wine. Attributes related to sensory quality, such as "flavour intensity", "colour intensity", "extract", "astringency", are predominantly included in Principal Component 1, with a variance of 93.84%, while "acidity", " "bitterness" and "sweetness" are equally included in both PC1 and PC2 components. This result shows that PC1 could be considered the axis that includes the most quality-related sensory components, and therefore the order of quality of the wines evaluated, according to these criteria, is, as follows, from the one with the highest quality: FN20-ChiBo, FN20-Bord, FN20-Chit. The sensory analysis on the OIV score sheet shows (Table 3) that the order of the wines according to the sensory quality is also: FN20-ChiBo (84.33 points), FN20-Bord (82.00 points), FN20- Chit (80.67 points).

Figure 2b shows that, for flavour categories, the evaluators determined that the floral aromatic notes are more pronounced in the FN20-Bord variant, the spicier ones in FN20-Chit and FN-ChitBo, and the fruity ones being relatively equally represented in all variants. Obviously, this is a simplification of the aromatic complexity of a wine, but it is known that PCA analysis reduces the number of complex independent variables and embodies them in 2 variables, PC1 and PC2, which depend on all the original independent variables, to a smaller or to a greater extent. Therefore, it should not be understood that the samples do not contain flavours from all the above categories, but the ratio between them makes the perception more inclined in the direction of a category or combination, depending on the sample being evaluated.

3. Specificities regarding the colour of wines

The colour of Fetească neagră wines obtained with various interventions in the organic culture technology was evaluated with the help of spectrophotometry.

In order to evaluate the colour specificities, the CIEL*ab* parameters were obtained, to effectively determine the degree of red (parameter *a*) and yellow (parameter *b*) contained in the wine colour, as well as the brightness L, shade *h* and chromaticity *c*. The average results and standard errors are shown in Table 4 for all determined colour parameters.

Table 4. Results of the colour parameters evaluation and total polyphenolic index of experimental organic wines using spectrophotometry

CIEL*ab* parameter	FN20-Bord	FN20-Chit	FN20-ChiBo
L	56.51 ± 0.20[b]	**60.19 ± 0.15**[a]	56.49 ± 0.18[b]
a	**37.89 ± 0.26**[b]	33.66 ± 0.20[a]	36.01 ± 0.25[c]
b	7.98 ± 0.18[b]	**9.23 ± 0.18**[a]	7.57 ± 0.19[c]
c_{ab}	**38.72** ± 0.2 [b]	34.90 ± 0.18[a]	36.80 ± 0.23[c]
h_{ab}	0.21 ± 0.0 [b]	**0.27** ± 0.04[a]	0.21 ± 0.04[b]
IPT	45.27 ± 0.10[b]	**51.64** ± 0.18[a]	50.36 ± 0.12[c]

*Different letters show that there is a significant difference 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 in bold.

It is thus observed that the FN20-Chit sample has the highest degree of transparency, the difference in brightness compared to the other two samples being statistically significant. This may indicate that FN20-Chit is more physically and chemically stable than the other samples.
Regarding the colour parameters *a* and *b*, we can appreciate that the control is significantly redder than the samples with chitosan, the treatment with the latter apparently being correlated with an increase in the degree of yellow in the final colour of the wine. The FN20-ChiBo wine sample shows intermediate values for the red colour, being placed between FN20-Bord and FN20-Chit. The chromaticity or colour saturation, the c_{ab} parameter, follows the same behaviour as the red colour (parameter *a*), FN20-Bord having the most vivid colour, followed by FN20-ChiBo and then FN20-Chit.
In contrast, the colour shade, the h_{ab} parameter, correlated with the presence of yellow (parameter *b*), shows that FN20-Chit has a higher yellow/brown shade, while FN20-ChiBo and FN20-Bord have similar shades, without significant differences. This behaviour of the FN20-Chit sample, with a colour showing a higher yellow participation, is clearly correlated with some oxidizable phenols and the fact that the total polyphenol index is also significantly higher in this sample (Table 1 and Figure 5).
To determine the colour differences that occur in samples from chitosan-treated vineyards, the differences from the FN20-Bord control sample as well as the total colour difference, ΔE, shall be calculated for each parameter. The results are presented in Table 5.

Table 5. Differences in colour parameters of samples with chitosan treatments compared to control samples with Bordeaux mixture FN20-Bord

Differences from FN20-Board	Δ FN20-Chit	Δ FN20-ChiBo
ΔL	3.68	-0.02
Δ*a*	-4.23	-1.88
Δ*b*	1.25	-0.42
ΔC*ab*	-3.82	-1.92
Δh*ab*	7.82	2.72
ΔE	**5.75**	**1.92**

Compared to the control sample, the samples from the chitosan-treated vineyards are slightly more transparent (ΔL=3.7) and less coloured (Δ*a*=-4.2; ΔC_{ab}=-3.8) the total colour difference being much greater than 1 (ΔE=5.75), clearly showing that the sample is visibly different. Samples from vineyard plots treated with combinations of chitosan and Bordeaux mixture also show a visibly significant colour difference compared to the control treatment only with Bordeaux mixture, but the colour difference compared to the control is not as high (ΔE=1.92) as in the case of chitosan treatment.
In order to have a clearer picture of the colour of the experimental samples, they can be placed in a two-dimensional space described by parameters *a* and *b* (Figure 3).

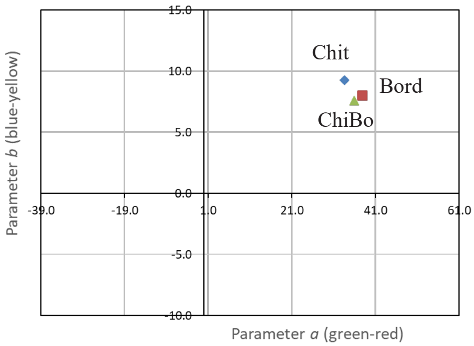

Figure 3. Placement of experimental samples in the two-dimensional colour field parameter *a* vs. parameter *b*

All wines are placed, as expected, grouped in the red-yellow region of the chromatic space. In such a two-dimensional chromatic space (parameter *a* vs. parameter *b*), which does not take into account the brightness and chromaticity of the colour, all samples are grouped in the same region, the apparent colour being a combination of red and yellow. Thus, this diagram clearly demonstrates that the FN20-Border control samples are redder than the others (shift to the right) and the FN20-Chit is yellower than the others (upward movement). Therefore, we can say that the treatments on the vines also influenced the colour of the wines, very possibly by the presence of phenolic compounds (tannins) in higher concentrations in the samples from vines with chitosan treatments.
As for the total polyphenol index (TPI) for our Feteasca neagră wines, which do not have a high phenol load compared to other red wines such as Cabernet Sauvignon, Syrah or Merlot, the values were between 45 and 52 absorbance units (Table 1 and Figure 4).

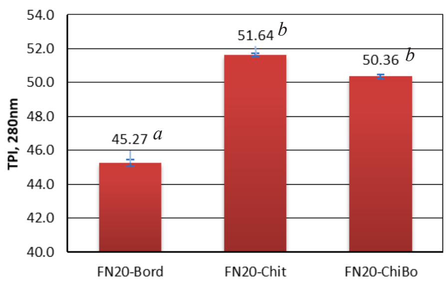

Figure 4. The index of total polyphenols (TPI) for the organic wines obtained with interventions in culture technology

The TPI shows that the treatment with chitosan, both alone and in combination with Bordeaux mixture, leads to an increase in the concentration of phenols in grapes, which is very beneficial for red wines.

CONCLUSIONS

The study shows that the chitosan treatment tends to induce a different and more pleasant sensory profile than that of the control with Bordeaux mixture.

The fact that chitosan treatments in vines bring changes in the chemical composition of plants and grapes obviously has its mark on the quality of the wine, the observed effects on the sensory qualities, aroma and colour being beneficial.
The effect of increasing the total polyphenol content of grapes / wine from vineyards treated with chitosan was also observed in our study.
Consequently, chitosan treatments appear to improve the phenolic content and aromatic profile of the resulting wines, but other experiments of this type still need to be performed until this practice can be recommended.

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REFERENCES

Adamuchio-Oliveira, L.G., Mazaro, S.M., Mógor, G., Sant'Anna-Santos, B.F., Mógor, A.F. (2020). Chitosan associated with chelated copper applied on tomatoes: enzymatic and anatomical changes related to plant defense responses, *Scientia Horticulturae*, *271*. 109431.

Antoce, O.A., Namolosanu, C.I. (2007). Method of creating a sensory profile for defining and evaluating the typicality of wines, Patent Invention 123129 issued by OSIM on 30.11.2010.

Apetroaei, M. R., Zgarian, R. G., Manea, A.-M., Rau, I., Tihan, G. T., & Schroder, V. (2016). New source of chitosan from Black Sea marine organisms identification. *Molecular Crystals and Liquid Crystals*, *628*(1), 102–109.

Artem, V., Antoce, O.A., Geana, E.I., Ionete, R.E. (2021a). Study of the impact of vine cultivation technology on the Feteasca Neagra wine phenolic composition and antioxidant properties, *J Food Sci Technol.*, Springer, *42*. 1–12.

Artem, V., Antoce, O.A., Geana, E.I., Ranca, A. (2021b). Effect of grape yield and maceration time on phenolic composition of 'Fetească neagră' organic wine. *Not. Bot. Horti Agrobot*., *49*(2), 1–10.

Artem, V., Ranca, A., Nechita, A., Tudor, G., Iliescu, M., Antoce, O.A. (2020). Influence of the bud load on the quality of grapes and wines obtained from Cabernet Sauvignon. *Journal of Environmental Protection and Ecology*, *21*(1), 142–150.

Bittelli, M., Flury, M., Campbell, G.S., Nichols, E.J. (2001). Reduction of transpiration through foliar application of chitosan, *Agricultural and Forest Meteorology*, *107*(3), 167–175.

Dagostin, S., Ferrari, A. & Pertot, I. (2006a). Efficacy evaluation of biocontrol agents against downy mildew for copper replacement in organic grapevine production in Europe. *Integrated Protection in Viticulture, IOBC/wprs Bulletin*, *29*(11), 15–21.

Dagostin, S., Vecchione, A., Zulini, L., Ferrari, A., Gobbin, D. & Pertot, I. (2006b). Potential use of biocontrol agents to prevent Plasmopara viticola oospore germination. *Integrated Protection in Viticulture, IOBC/wprs Bulletin*, *29*(11), 43–46.

Dagostin, S., JakobSchärer, H-J., Pertot, I. & Tamm L. (2011). Are there alternatives to copper for controlling grapevine downy mildew in organic viticulture? *Crop Protection*, *30*(7), 776–788.

Gutiérrez-Gamboa, G., Pérez-Álvarez, E.P., Rubio-Bretón, P., Garde-Cerdán, T. (2019). Changes on grape volatile composition through elicitation with methyl jasmonate, chitosan, and a yeast extract in Tempranillo (*Vitis vinifera* L.) grapevines, *Scientia Horticulturae*, *244*. 257–262.

Iriti, M., Vitalini, S., Di Tommaso, G., D'amico, S., Borgo, M., Faoro, F. (2011) New chitosan formulation prevents grapevine powdery mildew infection and improves polyphenol content and free radical scavenging activity of grape and wine, *Australian Journal of Grape and Wine Research*, *17*(2), 263–269.

Kim S.K. (2010). Chitin, chitosan, oligosaccharides and their derivatives: Biological activities and applications, CRC Press.

Lo'ay, A, A., El-Khateeb, A.Y. (2018). Impact of chitosan/PVA with salicylic acid, cell wall degrading enzyme activities and berries shattering of 'Thompson seedless' grape vines during shelf life, *Scientia Horticulturae*, *238*. 281–287.

Ma, J., Faqir, Y., Tan, C., Khaliq, G. (2022). Terrestrial insects as a promising source of chitosan and recent developments in its application for various industries, *Food Chemistry*, 373(A).

OIV (2021). OIV standard for international wine and spirituous beverages of vitivinicultural origin competitions, Available online: https://www.oiv.int/public/medias/7895/oiv-patronage-competition-norme-ed-2021.pdf (accessed on July 2021).

OIV (2021). International Compendium of Methods of Analysis of the OIV. Available online: https://www.oiv.int/en/technical-standards-and-documents/methods-of-analysis/compendium-of-international-methods-of-analysis-of-wines-and-musts (accessed on July 2021).

Oladzadabbasabadi, N., Nafchi, A.M., Ariffin, F., Wijekoon, M.M.J.O., Al-Hassan, A.A., Ali Dheyab, M., Ghasemlou, M. (2022). Recent advances in extraction, modification, and application of chitosan in packaging industry, *Carbohydrate Polymers*, 277, 118876.

Vitalini, S., Gardana, C., Zanzotto, A., Fico, G., Faoro, F. Simonetti, P., Iriti, M. (2011). From vineyard to glass: Agrochemicals enhance the melatonin content, total polyphenols and antiradical activity of red wines, *Journal of Pineal Research*, *51*. 278–285.