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IMPROVEMENT OF THE RED WINES QUALITY BY USING YEAST DERIVATIVES AS AN ALTERNATIVE TO LEES AGING

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Abstract
The macromolecules originating from yeast play an essential function in the colloidal equilibrium of wines. For several years now, mannoproteins from alcoholic fermentation and yeast autolysis have been studied to improve tartaric balance and stabilize red wine color and phenolic compounds. Thus, a commercial yeast derivatives product was applied (30 g/hL) during maceration, compared with the traditional wines aging on the lees, to evaluate the influence of treatment during alcoholic fermentation on wine's chromatic and sensory characteristics after 3 and 6 months of aging. The yeast derivatives product enhanced the structure and volume of red wines during maturation and reduced the feeling of dryness, attenuating tannins with aggressive structure. The addition of yeast derivatives enriched the red wine in colloids and nitrogen compounds (amino acids, peptides, low molecular weight proteins) during the fermentation, significantly improving the sensory profile and protein stability. From a sensorial point of view, the wine treated with yeast derivatives was more equilibrated, having expressive characteristics such as roundness and fullness. Overall compared to traditional aging on lees, the commercial treatment was more stable, eliminating the risk of unwanted sensory deviations such as hydrogen sulfide odors.

Keywords: red wine, yeast derivatives, lees aging, chromatic characteristics

Introduction

Yeast derivatives represent a class of compounds with multiple beneficial effects in winemaking in different steps such as alcoholic fermentation, malolactic fermentation, and aging period (Pozo-Bayón *et al.*, 2009).

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In enology, the yeast derivatives involved (cell walls, protein derivatives, low molecular weight nitrogen compounds, polysaccharide derivatives) present a different mode of action (Croitoru, 2009). They have the ability to improve the alcoholic process of musts and the malolactic fermentation process of wines. Thus, the mechanisms by which they include two types: a) a mechanism of detoxification for the fermentative environment as a result of the adsorption of saturated fatty acids with medium-chain (with 8, 10, and 12 carbon atoms), which are known as inhibitors for cellular activity; and b) a mechanism to activate fermentation processes through an additional intake of long-chain unsaturated fatty acids such as oleic acid (with 18 carbon atoms and a double bond in the first position) (Croitoru, 2009).

Yeast derivatives are usually used in enology as protectors during yeast rehydration and improve alcoholic and malolactic fermentation (Soubeyrand *et al.*, 2005; Feuillat and Gerreau, 1996; Díez *et al.*, 2010). Also, they are used as a traditional alternative for wine aging on lees with multiple advantages. The major advantage is the rate of mannoproteins release, which is superior to conventional aging via the lees technique with a high impact on the sensory quality of the wine (Del Barrio-Galán *et al.*, 2011, 2012 a, b; Guadalupe *et al.*, 2010). The effect is due to the interaction of the phenolic compounds from the wine with mannoproteins resulting in very stable polymeric structures which do not interact with salivary proteins from the mouth, improving the mouthfeel properties of wine (Li *et al.*, 2017, 2018).

The addition of low molecular weight nitrogen compounds in wines refers, in fact, to glutathione addition. The presence of molecular oxygen has a detrimental effect on yeast cells due to intracellular protein oxidation (the process known as "cell aging"). During cell multiplication, to combat this oxidative process, the yeast cell uses all the glutathione present inside as a reserve resource against possible deficiencies caused by the presence of oxygen. For this reason, the addition of glutathione-rich yeast derivatives rebalances the cell composition and eliminates the danger of oxidation (Croitoru, 2009).

Moreover, the influence of the addition of polysaccharide derivatives refers to the detoxification phenomenon by precipitating the remaining substances from the phytosanitary treatments applied to the vines.

The influence of yeast derivatives products addition on alcoholic fermentation stimulates cell multiplication and fermentation kinetics for equivalent concentrations of amino nitrogen because these activators have a much more complex composition than classical fermentation activators (González-Royo *et al.*, 2017). The influence of yeast derivatives products addition on malolactic fermentation kinetics refers to a favorable effect to accelerate the catabolism of malic acid by lactic bacteria (Mekoue-Nguela *et al.*, 2016). The contribution of yeast derivatives products to the improvement of the sensory properties of wines concerns both the visual and the olfactory and gustatory aspects and the influence on their global sensory evaluation (Rodríguez-Bencomo *et al.*, 2010).

This study aimed to evaluate the influence of treatment with a product obtained from yeast cell walls rich in polysaccharides and mannoproteins during alcoholic fermentation on wine's chromatic and sensory characteristics after 3 and 6 months of wine aging.

Materials and methods

The plant material used was grapes from the Fetească neagră variety harvest of 2020, cultivated in the Dealul Bujorului vineyard, located in southeast Romania. The grapes were manually harvested at full maturity and then were carefully transported to the winery.

Winemaking process

The grapes were manually destemmed and crushed (manual crusher), and then the mash was sulphited with 50 mg/hl of SO_2 and distributed in fermentation tanks of 100 L capacity. The fermentation process has been performed by inoculating the tank with dry yeast *Saccharomyces cerevisiae* var. *cerevisiae* (Fermactive Rouge Expression, AEB Spindal, France) using a dose of 25 g/hL. The must fermentation has been performed in contact with the grape skins and seeds. The fermentation temperature ranged between 22 and 25 ºC. After five days of fermentation, the free-run wine was gravitationally separated, and the resulting pomace was pressed using a manual press. Both free run and pressed wines were mixed and stored in 50 L tanks to complete the alcoholic fermentation at the temperature of 17-18 ºC. The alcoholic fermentation was considered at the end when the wine density was less than 999 g/L. The fermentation tanks were divided into three groups of three tanks each. First, three tanks were considered controls (V1) where the wines at the end of the spontaneous malolactic fermentation were separated from the lees. In the next three tanks, the addition of 30 g/hL Fermactive® Elevage RV (Sodinal, Romania) during maceration has been performed (V2), whereas, in the last three tanks (V3), the wine aging has been performed on the lees. Fermactive® Elevage RV is rich in nitrogenous substances, vitamins, and natural growth factors. All samples were analyzed after the end of malolactic fermentation and after 3 and 6 months of aging.

Chromatic parameters estimation

The chromatic parameters of the wines were determined spectrophotometrically (Libra S22, Biochrom, UK).

The Glories parameters (Glories, 1984) were estimated using optical densities of wine samples at the three wavelengths (420 nm, 520 nm, 620 nm). Based on these values 2 parameters were calculated:

$$\text{Color intensity: } CI = OD_{420nm} + OD_{520nm} + O_{620nm} \quad (1)$$

$$\text{Hue or color tint: } T = OD_{420nm}/OD_{520n} \quad (2)$$

Also, the proportion of red color produced by the flavylium cations of the free and bound anthocyanins (dA%) was estimated according to Glories (1984), using the following formula:

$$dA(\%) = \left[- \left(\frac{OD_{420nm} + OD_{620nm}}{2} \right) \right] \times \left(\frac{1}{OD_{520nm}} \right) \times 100 \quad (3)$$

PVPP index

Polyvinylpolypyrrolidone (PVPP) index expresses the proportion of anthocyanins combined with tannins that are discolored by sulfur dioxide. The determination of the index requires the adsorption of phenolic compounds on PVPP, the selective elution of free anthocyanins using a hydroalcoholic solvent, and at the end, their estimation (Glories, 1984).

Gelatin index

Gelatin index was estimated according to Ribéreau-Gayon *et al*. (1998).

Total nitrogen content

The total nitrogen content was performed according to the method SR 6182-4:2009.

Sensorial analysis

A panel of 12 evaluators performed the sensory analysis of wine samples, all of them being certified as wine tasters. For the sensory evaluation, the following descriptors were used: color intensity, olfactory intensity, olfactory quality, gustatory intensity, gustatory quality, astringency, bitterness, roundness, and global appreciation. A hedonic scale of 9 points with scores from 1 (very dislike) to 9 (very like) was used.

Statistical analysis

All measurements were performed in triplicates, and the data are expressed as mean ± of the standard deviation.

Results and discussion

The influence of yeast derivatives addition on the chromatic characteristics of red wines

In a reducing medium, the intensity of the yellow color measured by the value of the optical density at the wavelength λ = 420 nm tends to decrease, while in an oxidative medium, an inverse tendency to increase the value was observed.

In our comparative experimental study, the variant V3 with traditional aging on lees had the highest value of OD_{420nm} parameter compared to the other two variants after 3 and 6 months of aging (Figure 1).

In the control variant V1 and the variant treated with the product Fermactive® Elevage RV (30 g/hL) variant V2, the value of OD_{420nm} parameter remained almost at the same value of 0.11 after six months of aging. This proves that wines treated with the yeast derivatives product were better protected from oxidation due to the presence of antioxidant compounds (peptides, amino acids with -SH groups) naturally present in the composition of this product.

The highest value of the OD_{420nm} parameter was recorded in variant V3, having a value of 0.153 after three months of aging and a value of 0.208 after six months of aging on lees. This can be explained by the oxidation process of polyphenolic compounds from wines during the aging on lees.

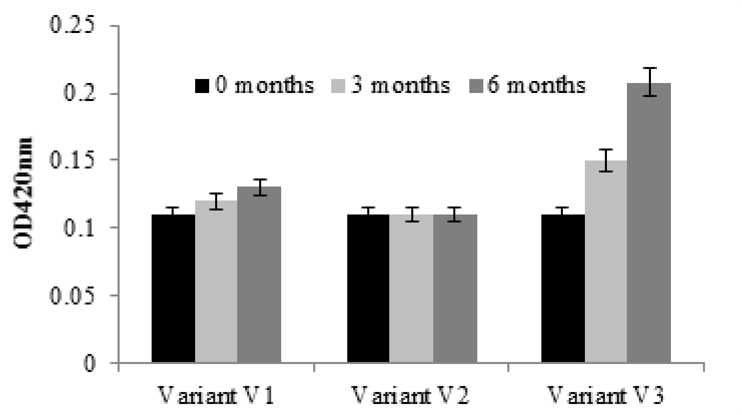

Figure 1. Evolution of the yellow color values (DO_{420nm}) of wine samples over six months of storage. V1 – control; V2 – wine maceration by adding Fermactive® Elevage RV; V3 - wine aging on the lees.

The constituents of the yeast derivatives product exert antioxidant properties, which allow the quinones to be fixed on their surface so that the wines keep their olfactory-gustatory freshness. The presence of low molecular weight nitrogen compounds such as amino acids and peptides (e.g., glutathione) found in high concentrations in yeast derivatives can fix the oxygen and keep the wines resistant to oxidation processes. The experiments have shown the effectiveness of using yeast derivatives products on the polyphenolic stabilization of wines.

When color intensity (CI), tint (T), and the proportion of red color produced by the flavylium cations of the free and bound anthocyanins (dA%) of wines were measured, an increase in the values of CI and (dA%) in the presence of yeast cell walls (variant V2 treated with Fermactive® Elevage RV (30 g/hL)) was observed. In contrast, the tint values remained approximately constant (Figures 2, 3, and 4).

The CI for variant V2 presented 2.25 and 2.53, respectively, after 3 and 6 months of aging. The lowest value of CI was measured for control variant V1, values of 1.75 and 1.65 after three months and six months of aging.

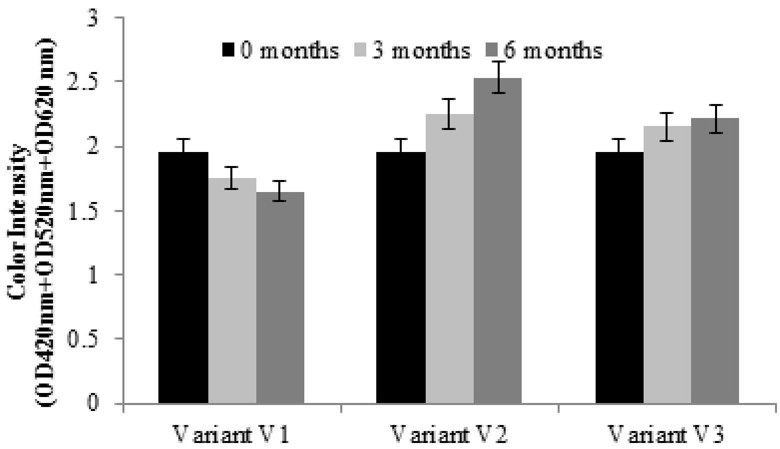

Figure 2. Evolution of the color intensity (CI) of wine samples over six months of storage. V1 – control; V2 – wine maceration by adding Fermactive® Elevage RV; V3 - wine aging on the lees.

On the contrary, the tint values (T) were lower for variant V2 treated with Fermactive® Elevage RV (30 g/hL), protecting the wine sample against oxidation. The measured values of tint were 0.60 and 0.61 after three months and six months of aging. The highest values of T were measured for variant V1 with values of 0.75 and 0.81 after three months and six months of aging.

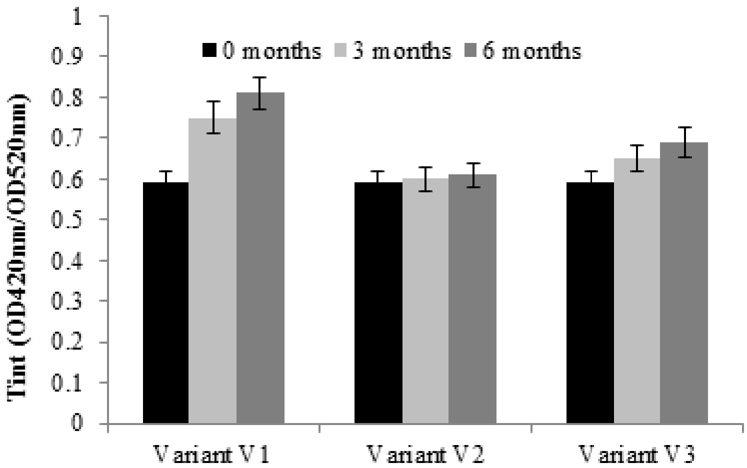

Figure 3. Evolution of the tint of wine samples over six months of storage. V1 – control; V2 – wine maceration by adding Fermactive® Elevage RV; V3 - wine aging on the lees.

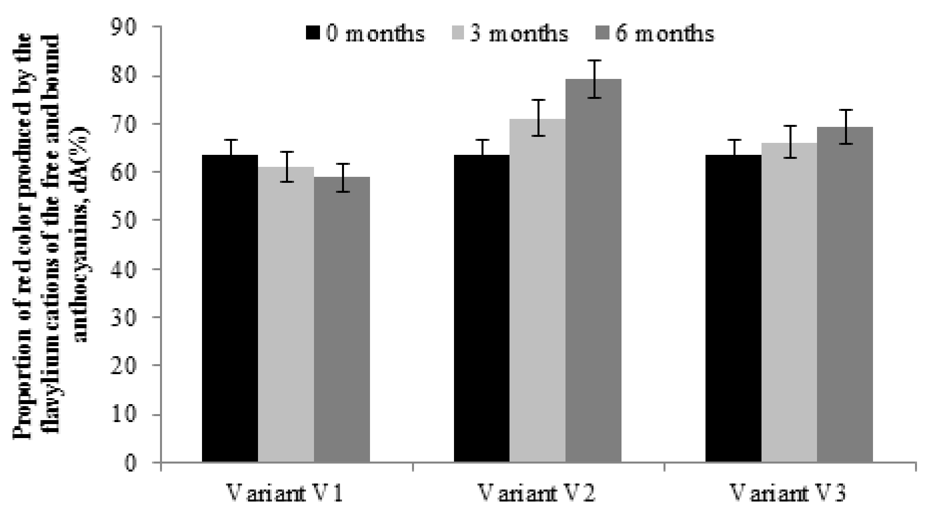

Figure 4. Evolution of the proportion of red color produced by the flavylium cations of the free and bound anthocyanins (dA%) of wine samples over six months of storage. V1 – control; V2 – wine maceration by adding Fermactive® Elevage RV; V3 - wine aging on the lees.

The highest values of dA were recoded for variant V2 with values of 71.2% and 79.3% after three months and six months of aging. Variant V1 presented a decreasing trend of the proportion of red color produced by the flavylium cations of the free and bound anthocyanins (dA%) from 63.5% to 61.1 after 3 months of aging and to 58.9% after 6 months of aging. This can be explained by the interaction between polysaccharides released from yeast derivatives product and polyphenolic compounds from the wine which may improve the wines color stability (Figures 3, 4, and 5) due to the formation of polymer pigments with higher stability which can inhibit the wine oxidation (Escot *et al.*, 2001; Francois *et al.*, 2007).

The estimated PVPP index of wine samples revealed higher values for variant V2 after 3 and 6 months of storage compared with other variants. The lowest value was determined for variant V1, with a value of 28.4 and 20.1 after three months and six months of aging (Figure 5). The results are in line with the ones reported by Kontoudakis *et al.* (2010) and Kontoudakis *et al.* (2011).

Gelatin index values reveal information on the proportion of the wine tannins that are able to be reactive with salivary proteins having a strong influence on astringency sensation in red wines. The values may vary between 25 to 80%.

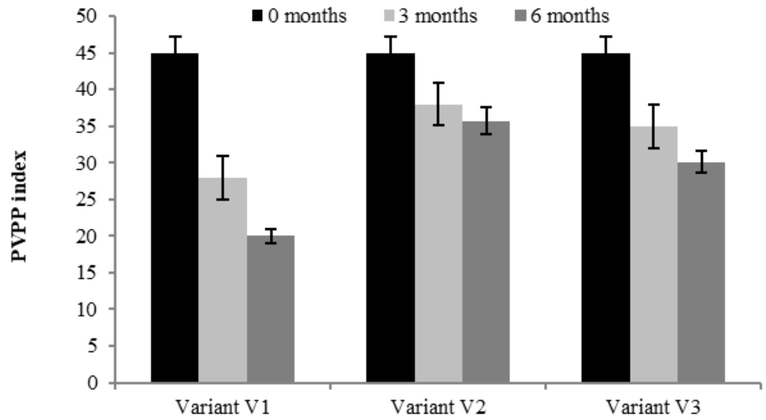

Figure 5. Evolution of the polyvinylpolypyrrolidone (PVPP) index of wine samples over six months of storage. V1 – control; V2 – wine maceration by adding Fermactive® Elevage RV; V3 - wine aging on the lees.

According to some researchers, a value higher than 60% measured for a wine indicates astringent tannins in the wine (Ribéreau-Gayon *et al.*, 1998; Arriagada-Carrazana *et al.*, 2005). The variant V2 presented a lower gelatin index value of 67 % after 3 months of aging and a value of 1% after 6 months of aging, indicating a lower initial astringency. These values were lower compared with variants V1 and V3. The tannins present in the variant V2 are less reactive than the protein fractions of gelatin, having a lower gelatin index (Figure 6). The control variant V1 displayed the highest gelatin index of 74% after 6 months of aging, indicating highly ionized anthocyanins and astringent tannins.

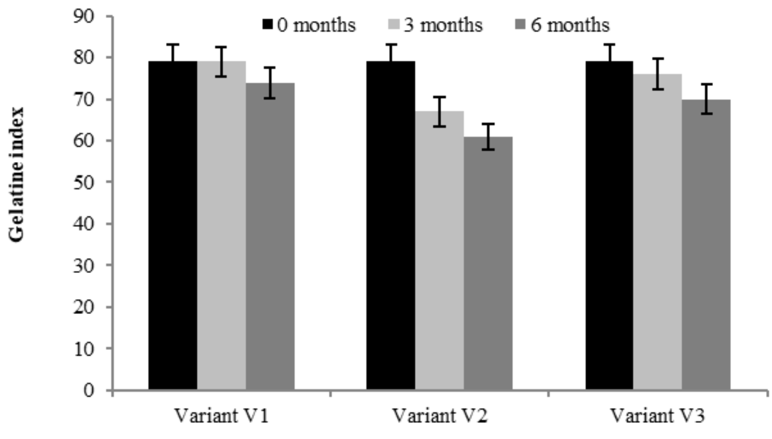

Figure 6. Evolution of the gelatin index of wine samples over six months of storage. V1 – control; V2 – wine maceration by adding Fermactive® Elevage RV; V3 - wine aging on the lees.

These results have confirmed that the yeast derivatives product addition induces a decrease in the gelatin index because the red wine astringency cannot be explained only by the chemical structure of the tannins but also by the incidence of combinations between tannins with compounds such as glycoproteins.

The influence of yeast derivatives addition on the total nitrogen content of wines

The enrichment of wine in total nitrogen content is one of the main results of the yeast autolysis process. During this process, an increase in the concentrations of amino acids, peptides, and proteins with low molecular weight due to the continuous release of these compounds from the cells in wine was observed.

As can be seen in Figure 8, variant V2 was the richest in total nitrogen content compared to other variants V1 and V3, having a value of 0.383 g/l after 3 months of aging and a value of 0.391 g/l after 6 months of aging. The variant V3 presented a value of 0.259 g/l after 3 months of aging and 0.275 g/l after 6 months of aging.

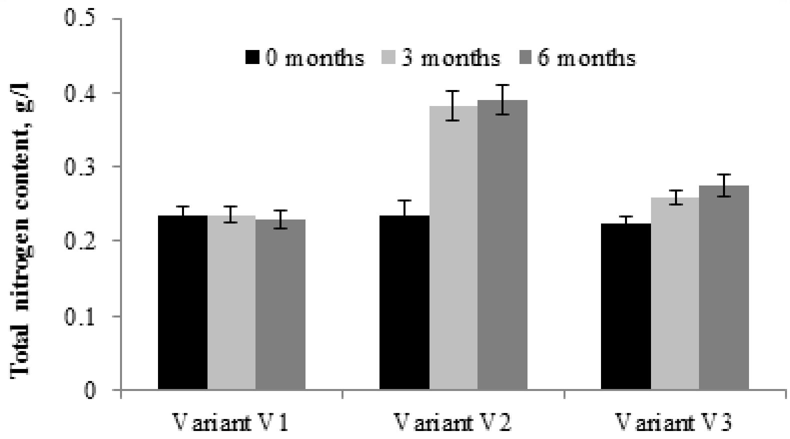

Figure 7. Evolution of the total nitrogen content of wine samples over six months of storage. V1 – control; V2 – wine maceration by adding Fermactive® Elevage RV; V3 - wine aging on the lees.

The influence of yeast derivatives addition on the sensory characteristics

By using yeast derivatives product addition in wines, the release of polysaccharides which increase the unctuousness and taste persistence characteristics has an important impact on the sensorial characteristics of treated wines.

Using the 30 g/hL of Fermactive® Elevage RV product, an increase in fruity character was observed in parallel with a decrease in unpleasant vegetal notes. An increased wine roundness, deeper tannin intensity, and less astringency correspond to a better sensory profile adapted to the actual demands of red wine consumers (Figure 8).

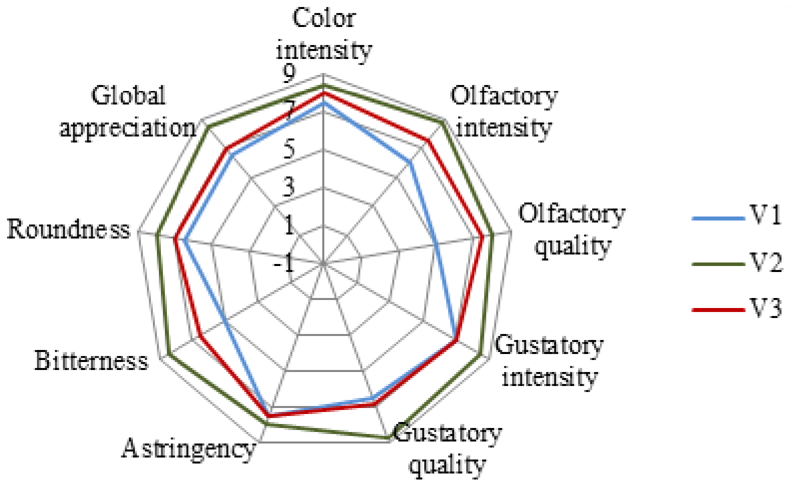

Figure 8. Sensorial profile of wine samples.

The contribution to the improvement of the sensory properties of wines concerns both the visual and the olfactory and gustatory aspects and the influence on their global sensory evaluation.

Several studies have reported the capability of yeast derivatives to interact with the polyphenolic compounds found in wines, having a high impact on their sensory characteristics (Mekoue-Nguela *et al.*, 2015 a, b).

Conclusions

Yeast derivatives products that are currently used in winemaking are efficient in the management of fermentation processes having a major contribution to the rate of fermentation and a high impact on the physicochemical and microbiological stability of wines.

The use of Fermactive® Elevage RV product in a dose of 30 g/hL results in a protective effect against oxidations, thus ensuring the protection of red wines' chromatic and sensorial characteristics. By using yeast derivatives product addition during fermentation, the wine is enriched in colloids and nitrogen compounds (amino acids, peptides, low molecular weight proteins), which significantly improves the sensory profile and protein stability.

From a sensorial point of view, the wine treated with yeast derivatives was more equilibrated, with expressive characteristics such as roundness and accentuated fullness.

Compared to traditional aging on lees, the treatment with Fermactive® Elevage RV product is more stable, avoiding the inconveniences related to the numerous re-suspensions of yeast sediments that may induce the risk of unwanted sensory deviations such as hydrogen sulfide odors.

Acknowledgments

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