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THE INFLUENCE OF THE *OENOCOCCUS OENI* MALOLACTIC BACTERIA IN MODELLING THE FLAVOR OF WHITE WINE

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Abstract: The research investigated the accumulation of malic acid and diacetyl in the white wines during the alcoholic fermentation of must with a *Saccharomyces cerevisiae* wine yeasts isolated by the author and *Oenococcus oeni* malolactic bacteria in a concentration of 10^6 CFU/mL. The two aroma compounds were detected and quantified in the resulting wines, making a comparison between the two technological systems, i.e.: co-inoculation of malolactic bacteria and sequential inoculation. Based on our determinations, it was ascertained that the *Oenococcus oeni* malolactic bacteria co-inoculation system in fermentative processes leads to a substantial reduction of malolactic bacteria and diacetyl concentrations. Thus, harmonious, balanced white wines are obtained, as specific to the area.

Keywords: malolactic bacteria, diacetyl, flavors, co-inoculation, *Saccharomyces cerevisiae*, alcoholic fermentation

INTRODUCTION

Must alcoholic fermentation is a very complex process; conducting and running this process is closely related to the flavor potential of the resulting wines. The aroma compounds of wines depend both on the variety of grapes from which the must was produced, and from the parameters established in order to conduct an optimal fermentative process, economic and quality wise. The microorganisms participating at these processes - both culture yeasts and bacteria - play an important role (Alexandre et al. 2004), (Arnink et al. 2005),

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(Antoce, 2007). If used in moderate quantities, malolactic bacteria impart a nice buttery flavor to the wine, as they, together with yeasts, participate in the production of secondary metabolites. During the alcoholic fermentation, malolactic bacteria, i.e. *Oenococcus oeni*, also take part in metabolizing sugars, malic acid and citric acid (Bartowski et al.2002), (Beelman et al. 1982), (Dick et al. 1992). Through alcoholic fermentation, yeasts synthesize diacetyl, which is subsequently metabolized in acetoin and 2, 3 butanediol. High concentrations of diacetyl (5-7 mg/L) in wines impart an unpleasant, unfit and inacceptable taste; these high concentrations are also connected to a high concentration of citric acid (Edwards et al. 1990),)Bartowski, 2008). Low concentrations (1-4 mg/L) of diacetyl contribute to a fresh butter or caramel flavor. These flavors can be stabilized by close monitorization of the concentration of *Oenococcus oeni* bacteria, combined with the culture yeasts used in winemaking, the selected yeasts having superior biotechnological properties (Mink et al. 2014). Recent studies have proved that the procedure of malolactic bacteria co-inoculation in fermentative processes contributes to the reduction of unpleasant compounds in wine, such as acetylated compounds. The way in which the fermentative process is conducted is important, as it depends on the type of yeast employed, as well as on the technological parameters established. An important factor is the contact time of wine with sediments of residual yeast, but also the amount of SO_2 used to destroy unwanted microorganisms in these processes. A higher fermenting temperature (25°C) leads to the formation of diacetyl ($C_4H_6O_2$) in higher concentrations than in the case of a fermentation taking place at a temperature close to 18°C. This aspect must be taken into consideration, even if the fermentation period is shorter.
The present study aims at monitoring the accumulation of malic acid and diacetyl in the white wines from Apold. It is extremely important to monitor these compounds, as, depending on their concentrations in wines, they cause substantial changes in flavor.

MATERIALS AND METHODS

Materials used were:
- 2.5 L white grape must from Apold, harvested in 2014
- *Oenococcus oeni* malolactic bacteria *Viniflora Oenos* lyophilized cultures from the company Sodinal, isolated in the Chr-Hansen laboratories in Denmark (10^6 CFU/mL)
- *Saccharomyces cerevisiae* wine yeasts selected from the Apold wine region, cultures from the collection of the Food Engineering and Biotechnologies Research Center Microbiology Laboratory of the Sibiu Faculty of

Agricultural Sciences, Food Industry and Environmental Protection (abbreviated as SCA211, SCA367, SCA181, SCA455, SCA392)
Alcoholic fermentation took place at 18ºC, in five versions corresponding to the selected strains, and lasted for 15 days. In the first procedure, in the case of each version, we co-inoculated the *Oenococcus oeni* strain 24 hours from the triggering of the alcoholic fermentation. Fifteen days later, we measured the amount of malic acid and diacetyl in the resulting wines. The second procedure used was the identification of the concentration of malic acid and diacetyl in the wines fermented with the above-mentioned strains, to we which we added the *Oenococcus oeni* strain at the end of the alcoholic fermentation. Fermentation took place in the microventilation system in the laboratory, in the the fermentator equipped with pH, temperature and CO_2, O_2 sensors.
The β-naphtol microcolorimetric method with was employed to determine malic acid (Târdea, 2007). The principle of this method is to separate malic acid as calcium malate. Sulfuric acid turns it into maleic anhydride, which condenses with B-naphtol, forming yellow-orange compounds. A Cecil 2010 Spectrophotometer was used to measure absorbance of preparations at a wavelength of 420 nm. The results were read on the calibration curve and reported in mg/L tartaric acid.
The GC-MS method developed by Richelieu et al. (1997) was used to determine the concentration of diacetyl. This method involves the decarboxylation of α-acetolactic acid into acetoin and diacetyl in the presence of the Fe3+ catalytic activator and the phosphate buffer solution pH 7.
Different methods for the sensorial analysis of the results obtained can be used, as profile method or Principal Component Analysis (Mironescu et al., 2008). The profile method was used in this research, the wines flavor profiles being analysed.

RESULTS AND DISCUSSIONS

Figure 1 shows that the *Saccharomyces cerevisiae* wine yeasts used in the study to ferment grape must present different activity with regard to the concentration of malic acid it metabolizes.
The malic acid remaining in the wine depends both on the yeast strain used, and on the technique used to introduce the *Oenococcus oeni malolactic bacteria.* From the data obtained, it is ascertained that, through the malolactic bacteria co-inoculation technique (at the beginning of alcoholic fermentation), superior amounts of malic acid are assimilated, in the end presenting a maximum amount of 6.492 g/L.
Low values of malic acid (4.823 g/L) were found when using SCA211 *Saccharomyces cerevisiae* strains; these values lead to a balanced flavor

profile of the resulting wine. In the case of the sequential inoculation system, the values of malic acid increase, reaching a maximum value of 7.877 g/L, again in the case when the SCA211 *Saccharomyces cerevisiae* wine yeast strain was used. Comparing the two methods used, it can be said that the malolactic bacteria co-inoculation system results in a superior biosynthesis of flavors in wine, as malic acid concentrations are 35% lower than when employing the sequential inoculation system.

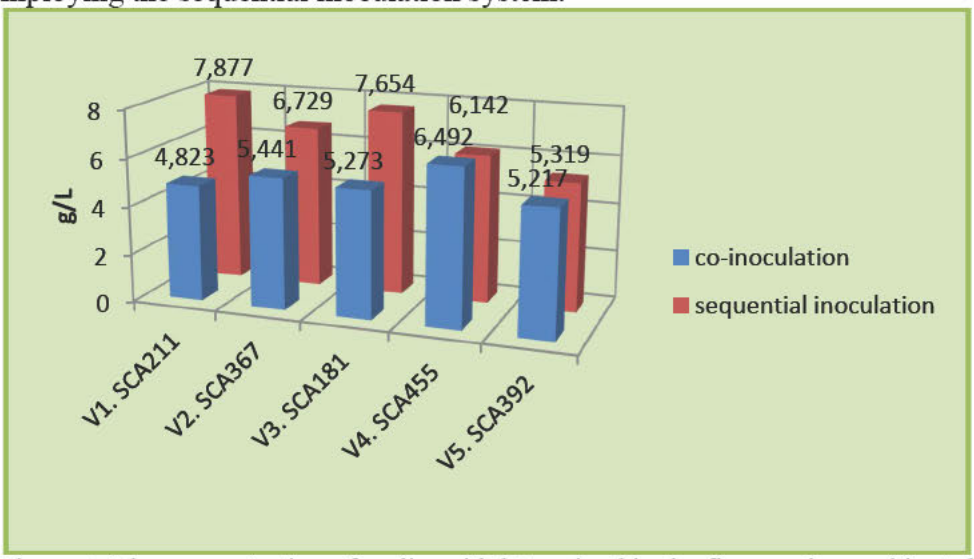

Figure 1. The concentration of malic acid determined in the five versions subjected to alcoholic fermentation (five *Saccharomyces cerevisiae* strains, yeasts SCA211, SCA367, SCA181, SCA455, SCA392, co-inoculation system and sequential inoculation of *Oenococcus oeni* malolactic bacteria)

The amount of diacetyl remaining in the wine is extremely important, because of its effect on the aroma features of the wine. Figure 2 shows a minimum accumulation of diacetyl of 1.832 mg/L when the SCA392 yeast strain was used in the co-inoculation system, followed by strain SCA181, with values of 1.917 mg/L, strain SCA211 with values of 2.793 mg/L. Maximum values of diacetyl detected in this system were recorded in the case of strains SCA367 and SCA455, where the amounts recorded reach 3.235 mg/L and 3.458 mg/L respectively. In the case of the sequential inoculation system, it is ascertained that the amount of diacetyl increases to values over the threshold of 9 mg/L. A maximum amount of diacetyl is recorded in the case of strain SCA181, with a value of 9.813 mg/L. A minimum is recorded in the case of strain SCA367, where we identified and quantified 6.649 mg diacetyl/L. Intermediate diacetyl values were recorded when using *Saccharomyces cerevisiae* yeast strains code SCA211 with 8.766 mg/L, SCA455 with 7.281mg/L and SCA392 with 8.911 mg/L, sequential inoculation with malolactic bacteria.

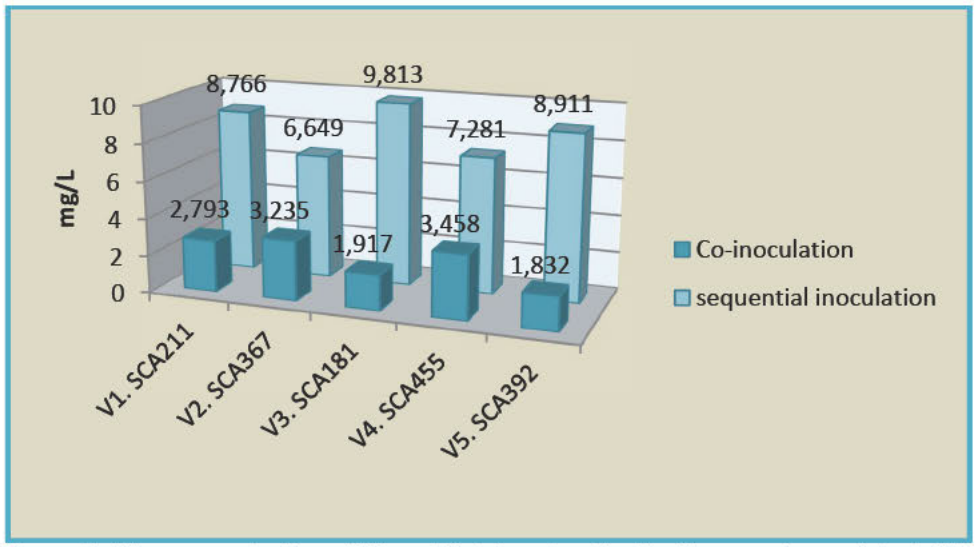

Figure 2. The concentration of diacetyl determined in the five versions subjected to alcoholic fermentation (five strands of *Saccharomyces cerevisiae* yeasts SCA211, SCA367, SCA181, SCA455, SCA392, co-inoculation system and sequential inoculation of *Oenococcus oeni* malolactic bacteria)

The values obtained led to the realization of the flavor profile of the resulting wines, as follows: Figure 3 shows the profile of the five wines resulting from the co-inoculation of *Oenococcus oeni* malolactic bacteria.

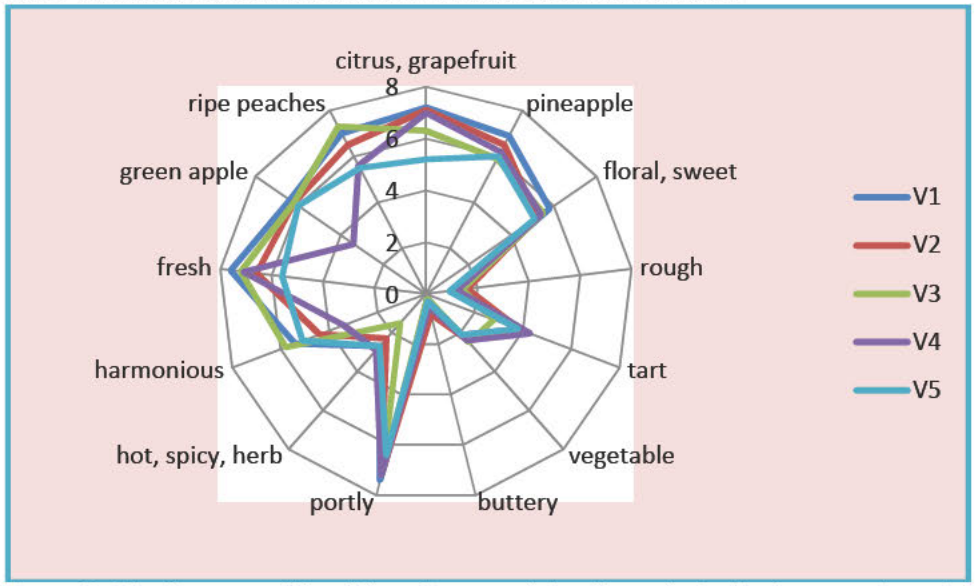

Figure 3. The flavor profile of the wines resulting from alcoholic fermentation with *Saccharomyces cerevisiae* wine yeasts SCA211, SCA367, SCA181, SCA455, SCA392, co-inoculation system with *Oenococcus oeni* malolactic bacteria

Figure 3 shows that the pleasant flavors characterizing the wines are similar, the notes obtained reflecting their qualities. Grades 1-3 present the very weak to weak flavors, grades 4-6 present typical, standard flavors, while grades 7-9 show predominant flavors. Characterizing these wines, we can state that they have predominant citrus notes: grapefruit, ripe peach; the resulting wines are less coarse, with fresh, harmonious notes.

Figure 4 shows the profile of the five wines resulting from sequential inoculation.

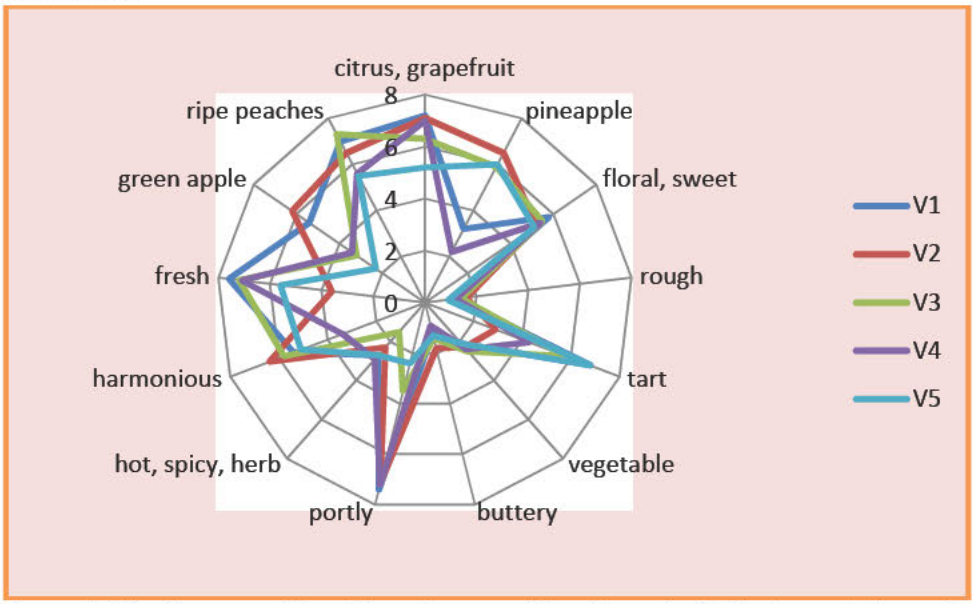

Figure 4. The flavor profile of the wines resulting from alcoholic fermentation with *Saccharomyces cerevisiae* wine yeasts SCA211, SCA367, SCA181, SCA455, SCA392, co-inoculation system with *Oenococcus oeni* malolactic bacteria

Figure 4 paints the picture of slightly sour, round-bodied wines, with ripe peach and green apple flavors, but also having pregnant buttery notes, which disfavors them.

Figure 5 shows that the grades representing the butter flavor is strongly differentiated between the two malolactic bacteria inoculation systems. In the case of the co-inoculation system, weak values are ascertained, which means that pleasant buttery notes persist, while, in the case of the sequential inoculation system, the strong taste of butter leads to a negative appreciation of the resulting wine flavor.

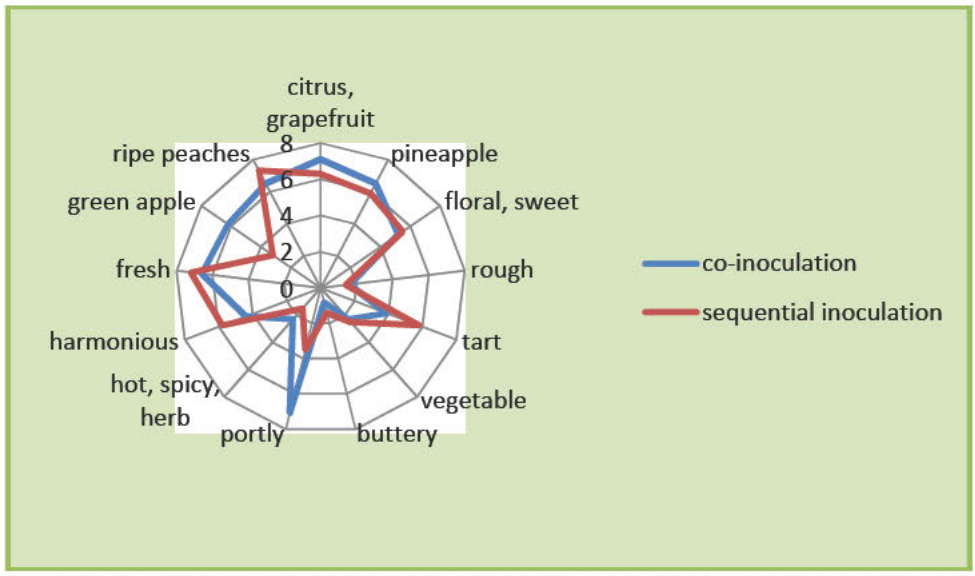

Figure 5. The flavor profile of the wines resulting from alcoholic fermentation with *Saccharomyces cerevisiae* wine yeast SCA181, co-inoculation system and sequential inoculation of *Oenococcus oeni* malolactic bacteria

CONCLUSIONS

Wine flavor is strongly influenced by the compounds resulting from the biosythesis of yeasts belonging to the *Saccharomyces cerevisiae* genus and the *Oenococcus oeni* malolactic bacteria participating in the alcoholic fermentation process. The type of yeast used leads to a diversification of flavors and to the potentiation of certain compounds in the wine, which can subsequently impart predominant notes. The use of malolactic bacteria in fermentative processes leads to a decrease of diacetyl concentrations in wines, especially when used in a co-inoculation system, i.e. 24 hours after the alcoholic fermentation was triggered. Employing sequential inoculation systems has little effect on the malic acid and diacetyl concentrations in wines, which recommends the use of this system. The yeasts selected to be used in these fermentative processes have shown superior flavor notes. The *Saccharomyces cerevisiae* SCA181 strain presented superior biotechnological qualities. The diacetyl concentration of wines depends on several factors. Nevertheless, the relation between the selected wine yeasts and the procedures regarding their cohabitation with malolactic bacteria during fermentative processes seem to be most important.

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