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# SYNTHESIS OF CARBONYL COMPOUNDS FROM *SACCHAROMYCES CEREVISIAE* STRAINS IN CABERNET SAUVIGNON WINES

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**ABSTRACT**

The ability of two *Saccharomyces cerevisiae* strains (Bordeaux, 8-11) to produce carbonyl compounds under different conditions of the alcoholic fermentation was investigated. The impact of the temperature and the inoculum yeast culture ratio on the synthesis of total aldehydes during the process was traced. A more pronounced effect of the temperature was observed. At the beginning of the fermentation, the total aldehydes rate increased rapidly and reached a maximum during the exponential phase of the yeast cells development (day 5$^{th}$), thereafter their amount began to go down. The strain *Saccharomyces cerevisiae* Bordeaux synthesized the most total aldehydes at a temperature of 24ºC, while *Saccharomyces cerevisiae* 8-11 - at 28ºC. Acetaldehyde, acetone and 2-butanone were identified in the experimental wines by gas chromatographic analysis. During the fermentation at a temperature of 20°C and 24°C, *Saccharomyces cerevisiae* Bordeaux produced more acetaldehyde, acetone and 2-butanone compared to 8-11. At 28°C *Saccharomyces cerevisiae* 8-11 produced more acetaldehyde and 2-butanone, 33.3% and 15% respectively, and 30% less acetone than *Saccharomyces cerevisiae* Bordeaux. The total aldehydes rate in the experimental samples did not have a negative effect on their aromatic and taste qualities.

**Keywords:** yeast, Cabernet Sauvignon, alcoholic fermentation, wine, aldehydes, sensory characteristic

## INTRODUCTION

The chemical composition of wine has been diverse, including numorous substances and compounds, some of which passed from the grapes, while others were formed in the process of the alcoholic fermentation, malolactic fermentation and storage.

From the group of the carbonyl compounds in the wine there were aldehydes (fatty, aromatic and furan) and ketones. Only traces, mainly of acetaldehyde, propionic and isobutyric aldehydes were found in grapes **(Chobanova, 2012)**. During the alcoholic fermentation, the yeast produced over 20 aldehydes from different precursors – amino acids, the respective alcohols, sugars. Most of them were formed as a result of the yeast metabolism, under the action of the alcohol dehydrogenase enzyme and the primary alcohol oxidation to aldehyde. There had been other mechanisms too – by enzymatic oxidation of alcohols by the yeast, by non-enzymatic oxidation of primary alcohols under the action of oxygen from the air, by oxidative deamination of amino acids and decarboxylation of keto acids, with the participation of quinones **(Bambalov, 1981; Romano *et al.*, 1994; Jackowetz *et al.*, 2011 b; Chobanova, 2012; Herzan *et al.*, 2020)**.

Their total amount varied from 10 to 200 mg/L, 90% of which had been acetaldehyde. It was the main volatile carbonyl component in wine that might be produced biologically (from yeast) or chemically (by oxidation). Of the acetaldehyde contained in the wine, only a small part originated from sugars. The rest had other paths of origin. It had been an intermediate product of alcoholic fermentation and was formed after decarboxylation of the pyruvic acid and before the ethanol synthesis. Other pathways of origin had been the self-oxidation of phenolic compounds to quinones with the evolution of $O_2$, which oxidized the ethyl alcohol to acetaldehyde, and the oxidative decarboxylation of valine under appropriate conditions **(Romano *et al.*, 1994; Chobanova, 2012; Mina and Tsaltas, 2017; Lago and Welke, 2019; Filimon *et al.*, 2021)**.

The acetaldehyde rate produced by the yeast changed during the alcoholic fermentation. At the onset of the process, its amount gradually increased, thereafter equilibrium occurred between its synthesis and its reduction to ethyl alcohol. Its concentration reached a maximum during the exponential phase of yeast cell development. Upon fermentation of 40-50% of the sugars its quantity began to drop down **(Bambalov, 1981; Jackowetz *et al.*, 2011 b; Orduña, 2014; Rosca *et al.*, 2016; Herzan *et al.*, 2020)**. That was due to its participation in the condensation processes with phenols or nitrogenous substances in the medium, its reduction to ethanol, its interaction with $SO_2$ and formation of a permanent aldehyde-bisulfite compound, interaction with the fermentation by-products **(Frivik and Ebeler, 2003; Swiegers *et al.*, 2005; Bueno *et al.*, 2018; Ferreira *et al.*, 2018; Lago and Welke, 2019)**.

The acetaldehyde concentration also affected the course of fermentation during the stationary phase of yeast development, when the alcohol content increased significantly. During this period, the supplement of acetaldehyde in the medium in high concentrations inhibited the process, while in moderate and low doses stimulated it. The acetaldehyde content could also be modulated by modifying the alcohol dehydrogenase catalytic reaction **(Roustan and Sablayrolles, 2002)**. While maintaining its viability yeast could reduce the amount of acetaldehyde during the last stages of fermentation and in prolonged contact with wine **(Jackowetz *et al.*, 2011 a)**.

Ketones in wine were also formed during the alcoholic fermentation by enzymatic oxidation of alcohols. They oxidized significantly more difficult, the reactions with $SO_2$ were very slow, binding about 65-85% of the keto compounds in wine. Their total amount could reach 40-60 mg/L mainly acetoin, diacetyl and γ-butyrolactone (**Rosca *et al.*, 2016; Mina and Tsaltas, 2017**). The concentration of acetone was 1-3 mg/L **(Chobanova, 2012)**.

The carbonyl compounds content in wines had been influenced by a number of technological factors such as degree of ripeness and composition of grapes, $SO_2$ content, the species and strain of yeast, fermentation conditions (temperature, aeration), pH of the medium, contact with air, heat treatment during vinification or storage **(Romano *et al.*, 1994; Jackowetz *et al.*, 2011 b; Chobanova, 2012; Rosca *et al.*, 2016; Lago *et al.*, 2017; Bueno *et al.*, 2018; Herzan *et al.*, 2020; Licek *et al.*, 2020; Filimon *et al.*, 2021)**. Yeast usually reduced acetaldehyde to ethanol, but under conditions of increased aeration and oxygen content in the medium, they could also perform the reverse reaction by oxidation.

The metabolic kinetics of acetaldehyde synthesis from *Saccharomyces* and non-*Saccharomyces* strains had been similar, however a significant variation in its amount was observed. Some non-*Saccharomyces* strains produced much less (*C. vini, H. anomala, H. uvarum, M. pulcherrima*) or much higher (*C. stellata, Z. bailli, S. pombe*) amounts compared to *Saccharomyces* strains **(Romano *et al.*,**

**1994; Li and Orduña, 2017)**. According to research of **Liu *at al.* (2016)** more aldehydes were produced in inoculated fermented wines than in spontaneously fermented wines which was mainly associated with decanal and benzeneacetaldehyde.
The acetaldehyde producing had been strongly affected by the fermentation temperature. According to most studies, it was enhanced when the temperature rised. Higher acetaldehyde rate was formed at higher fermentation temperature or pH **(Byrne and Howell, 2017)**. Its content at 30°C was significantly higher than at 18°C and 24°C, which might be due to the inhibitory effect of temperature on the activity of alcohol dehydrogenase that catalyzed the conversion of acetaldehyde to ethanol **(Romano *et al.*, 1994)**. According to **Jackowetz *et al.* (2011 b)**, however, lower fermentation temperatures were associated with the formation of higher concentrations of acetaladehyde, due to the lower degree of its conversion during the second phase of fermentation and the higher content of residual sugars.
Wines obtained without sulphitation of grape must contained small amounts of aldehydes. In the presence of $SO_2$ in the fermentation medium, aldehydes bound to it and accumulated in the wine. **(Bambalov, 1981; Romano *et al.*, 1994; Chobanova, 2012; Jackowetz *et al.*, 2011 a; Jackowetz *et al.*, 2011 b; Li and Orduña, 2017; Lago and Welke, 2019).** The addition of $SO_2$ to the grapes must increased production of acetaldehyde by the yeast**.** Acetaldehyde formation was a way of protecting yeast from the antiseptic effects of $SO_2$ **(Herzan *et al.*, 2020)**.
According to some studies, the amount of aldehydes in red wines was higher than in white wines, because after oxidation, phenolic compounds served as catalysts for the formation of acetaldehyde **(Chobanova, 2012)**. According to others, however, during malolactic fermentation, malolactic bacteria significantly reduced its amount by metabolizing acetaldehyde to ethanol. Terefore, its concentration in red wines was less than in white ones **(Jackowetz *et al.*, 2011 a; Orduña, 2014; Herzan *et al.*, 2020)**.
Carbonyl compounds had been one of the most reactive groups of wine substances. Their representatives, as well as the products of the reactions in which they participate, affected the wine composition and its organoleptic qualities **(Romano *et al.*, 1994; Chobanova, 2012; Liu *at al.*, 2016)**. Aldehydes and ketones were easily volatile substances with a specific flavor that determined their great influence for the wine aroma and bouquet. Fatty and aromatic aldehydes had a strong fruity aroma. Acetaldehyde, as the main aldehyde in wine, in low ratios had the smell of a green apple, freshly cut grass or walnuts but at a ratio over 100 mg/L gave an oxidized tinge **(Jackowetz *et al.*, 2011 b; Chobanova, 2012; Orduña, 2014; Byrne and Howell, 2017; Filimon *et al.*, 2021)**. Concetration of up to 100 mg/L of acetaldehyde and 1-4 mg/L of diacetyl could be described as desirable and contributing to the complexity of wine aroma (**Mina and Tsaltas, 2017**). Ketones also had a specific smell and could give the wine both pleasant and negative nuances. In high doses they negatively affected the aroma of wine, associated with oxidation **(Chobanova, 2012; Rosca *et al.*, 2016)**.
The purpose of the present research was to study the synthesis of aldehydes during the alcoholic fermentation, the carbonyl compounds content in Cabernet Sauvignon wines and their significance for wine sensory characteristics.

## MATERIAL AND METHODS

### Vinification

The investigation was carried out at the Institute of Viticulture and Enology (IVE), Pleven, Bulgaria. Grapes of Cabernet Sauvignon variety, cultivated at the experimental vineyards of the IVE, was used for the trials. The grapes were harvested at optimal technological maturity and processed according to the classic red dry winemaking technology under the conditions of microvinification **(Yankov, 1992)**.

### Alcoholic fermentation

The process occurred under the following conditions:

- 4.0 kg of grape pomace, sulfated with 50 mg/kg $SO_2$, with sugars content 23.10% and titratable acids 6.15 g/L;
- Inoculum - 48-hour active yeast culture of the strains *Saccharomyces cerevisiae* Bordeaux and *Saccharomyces cerevisiae* 8-11, in quantity of 2%, 3%, 4%;
- Fermentation temperature – 20ºC, 24ºC, 28ºC;
- Daily control of the process by monitoring the change in dry matter, measured with an Abbe refractometer to a constant value.

The dynamics of the total aldehydes during the fermentation was monitored on day 1$^{st}$, on day 5$^{th}$ (rapid phase), on day 10$^{th}$ (quiet phase) and after the malolactic fermentation. Their quantity was defined by the bisulphite method **(Ivanov *et al.*, 1979)**. The course of spontaneous malolactic fermentation was qualitatively monitored by paper chromatography for organic acids **(Kramer, 1961)**.

### Neural networks

The experimental results from all variants were modeled through neural networks of the Statistica 8 software package using a second order quasi-Newton algorithm describing the influence of time, fermentation temperature and inoculum yeast culture on aldehydes synthesis. The results were presented in the form of surfaces that described the experimental data with high precision **(Chen *et al.*, 2006, Nicoletti *et al.*, 2009)**.

### Chemical composition

All experimental wines were analyzed for the ratio of alcohol, vol. % (distillation method, Gibertini distillation apparatus with densitometer); sugars, g/L (Schoorl method); total aldehydes mg/L (bisulphite method) and pH (pH-meter) **(Ivanov *et al.*, 1979)**.
The content of carbonyl compounds (acetaldehyde, acetone, 2-butanone) in the experimental variants were identified and qualitatively determined by gas chromatographic analysis made with GC 8000 Top Series **(Yoncheva *et al.*, 2019)**. The volatile components were extracted from the wine by the "headspace" method **(Hrivňak *et al.*, 2004; Kružlicova *et al.*, 2006)**. Based on the peak area, the ratio difference in the component content between the different variants was calculated. The samples, obtained at 20°C and 2%, were considered to be the standard (100%). For comparing both strains, *Saccharomyces cerevisiae* Bordeaux strain was assumed for standard (100%).

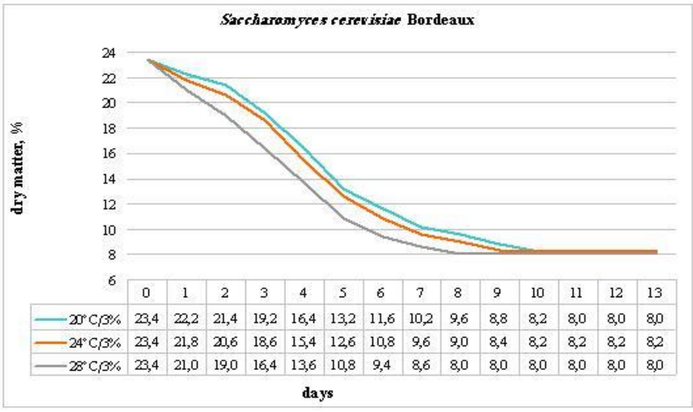
### Organoleptic profile

The sensory characteristics of the wines were evaluated by a 100-score system (Tsvetanov, 2001) by a 5-member tasting commission from the University of Food Technologies in Plovdiv, Bulgaria. All members of the tasting panel were experts – oenologists with long practice and experience in sensory analysis of wines. Mathematical modeling of the organoleptic analysis results was performed, presented by response surfaces.

## RESULTS AND DISCUSSION

### Alcoholic fermentation

When monitoring the course of the alcoholic fermentation, a similarity in the dry matter change was observed under the different process conditions. The studied strains showed high fermentation activity, as the intensity with which the fermentation began and the time for its completion were in correlation with the temperature and the quantity of inoculum yeast culture. At 20°C the fermentation started and proceeded more slowly. The studied strains demonstrated the highest activity at 28°C. With the increase of the inoculum yeast amount within one temperature range, fermentation started and ended earlier.
Figure 1 showed the change of dry matter during the alcoholic fermentation, taking place under the conditions of 3% active yeast culture inoculum and temperature 20ºC, 24ºC and 28ºC with the studied yeast strains. The results from following up the process in the other experimental variants were similar.



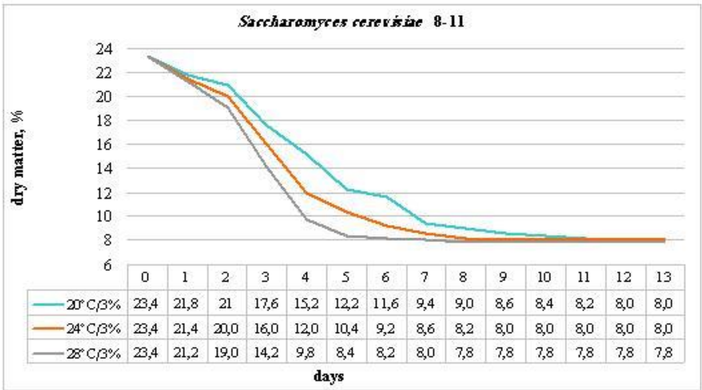
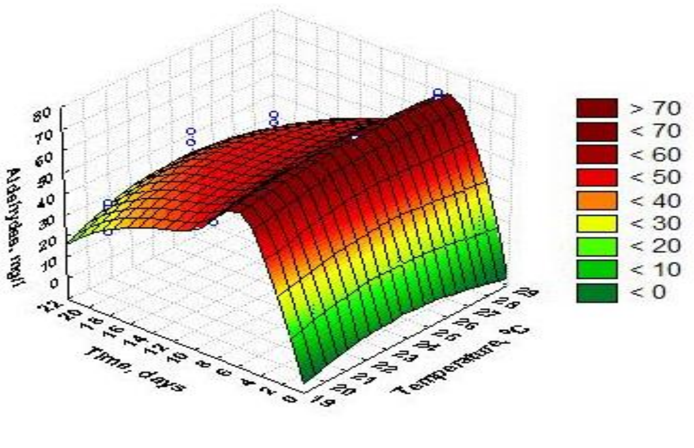



**Figure 1** Change of dry matter during the alcoholic fermentation with the studied strains *Saccharomyces cerevisiae*

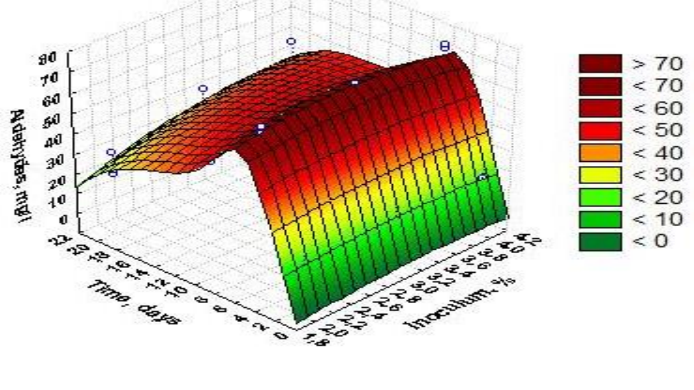
**Neural networks and total aldehydes accumulation during the alcoholic fermentation**

During the fermentation, the synthesis of total aldehydes from the strains was monitored, depending on the change of the fermentation parameters, temperature and amount of inoculum yeast culture. Figure 2 and figure 3 showed the dynamics of accumulation of their total amount during the process (day 1st, 5th, 10th, 20th). Neural networks were drawn up for all variants, modeling the processes and characterized by high accuracy of description of the experimental data. They demonstrated a stronger effect of the temperature than the amount of inoculum yeast culture.

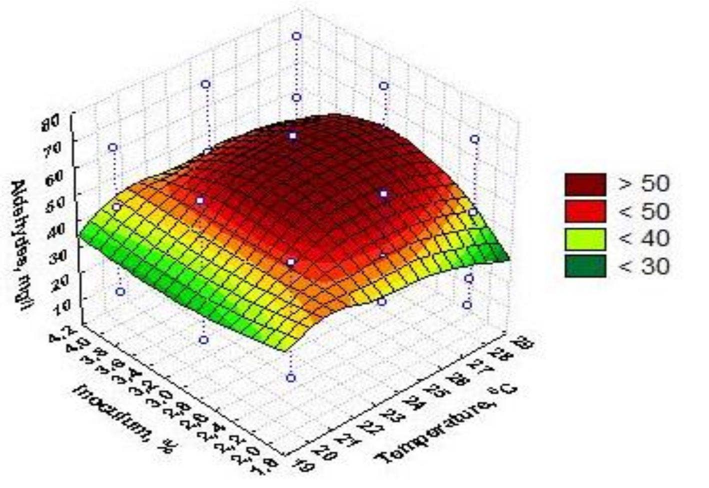
The results obtained, describing the total aldehydes change during the alcoholic fermentation, were similar to those found by other authors **(Bambalov, 1981; Swiegers *et al.*, 2005; Orduña, 2014)**. At the onset of the process, their rate increased rapidly and reached a peak during the exponential phase of yeast cell development (5th day). After the fermentation of most of the sugars in the middle of the process, their rate began to decrease (day 10th). That was due to their participation (mainly acetaldehyde) in the condensation processes or its reduction to ethanol and interaction with $SO_2$.




a)




b)



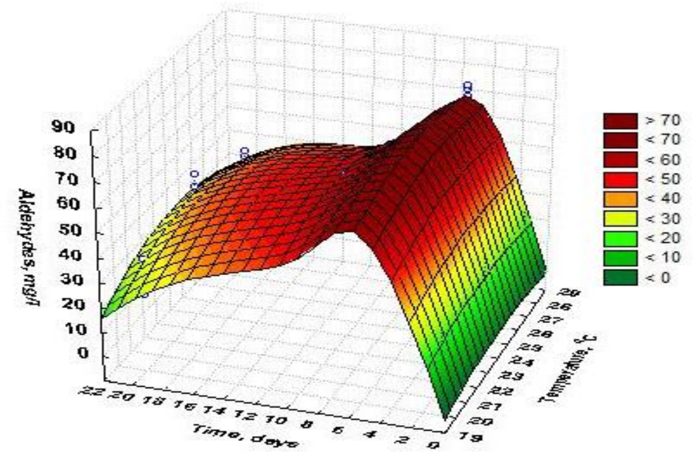

c)

**Figure 2** Change in total aldehydes during the alcoholic fermentation with the strain Bordeaux

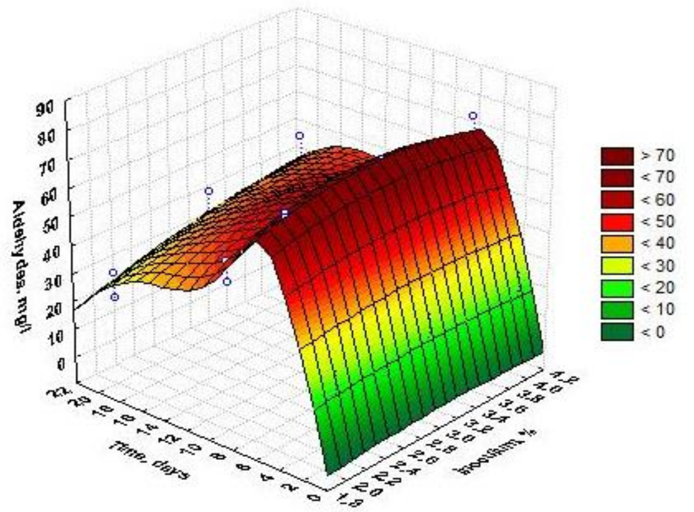
a) influence of temperature

b) influence of inoculum yeast culture amount

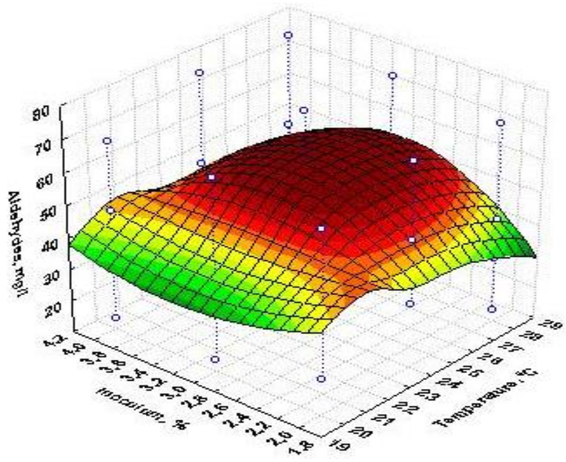
c) influence of temperature and inoculum yeast culture amount




a)




b)




c)

**Figure 3** Change in total aldehydes during the alcoholic fermentation with the strain 8-11
a) influence of temperature
b) influence of inoculum yeast culture amount
c) influence of temperature and inoculum yeast culture amount

In the conditions of this experiment there was no clear general trend of the studied yeast to synthesize the maximum amount at a certain temperature. The strains showed different ability to produce aldehydes depending on the conditions. No correlation between the temperature and the concentration of the tested metabolite was observed in the samples fermented with the strain *Saccharomyces cerevisiae* Bordeaux. Most total aldehydes were produced at 24°C – from 78.68 to 83.20 mg/L (day 5$^{th}$) and from 48.95 to 56.88 mg/L (day 10$^{th}$), respectively. *Saccharomyces cerevisiae* 8-11 produced the highest amount of aldehydes when the fermentation occurred at 28ºC – from 78.00 to 85.60 mg/L (day 5$^{th}$) and from 55.36 to 62.72 mg/L (day 10$^{th}$). With both strains within one temperature range when increasing the amount of yeast culture the rate of total aldehydes went up.

The results modeled by means of the neural networks confirmed that trend. The presented surfaces described their rate in the samples during the rapid phase under the individual and overall impact of the studied fermentation factors (Fig. 2, 3). In the strain *Saccharomyces cerevisiae* Bordeaux, the peak was observed in the variant 24ºC/4% (83.20 mg/L), and in the strain 8-11 in 28ºC/4% (85.60 mg/L). The minimum for both strains was at 20ºC/2% - 66.85 mg/L (Bordeaux) and 68.20 mg/L (8-11), respectively. The stronger effect of temperature on the fermentation process and the wine components was more pronounced in 8-11 than in Bordeaux.

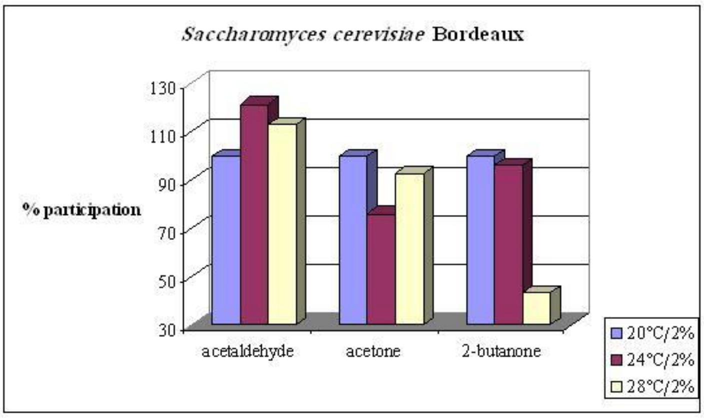
**Chemical composition**

The chemical composition of the obtained wines, after the alcoholic and malolactic fermentation, in terms of the alcohol, sugars and total aldehydes content, was presented in table 1. No significant contrast was found in the total aldehydes concentration produced by both strains in the wines. In the samples of the *Saccharomyces cerevisiae* Bordeaux their amount varied from 24.46 to 46.70 mg/L, and in the samples of *Saccharomyces cerevisiae* 8-11 – from 22.24 to 53.38 mg/L. The strain *Saccharomyces cerevisiae* Bordeaux synthesized the most at a temperature of 24ºC, while *Saccharomyces cerevisiae* 8-11 strain at 28ºC.

**Table 1** Chemical compositions and total aldehydes concentration in the experimental wines obtained with the studied strains *Saccharomyces cerevisiae*

| Variants | 20ºC | | | 24ºC | | | 28ºC | | |
|---|---|---|---|---|---|---|---|---|---|
| Indicators | 2% | 3% | 4% | 2% | 3% | 4% | 2% | 3% | 4% |
| | *Saccharomyces cerevisiae* Bordeaux | | | | | | | | |
| Alcohol, vol. % | 12.75 | 12.95 | 12.72 | 12.84 | 12.72 | 12.81 | 12.74 | 12.76 | 12.80 |
| Sugar, g/L | 2.35 | 1.81 | 2.18 | 1.98 | 2.01 | 2.22 | 2.18 | 2.35 | 2.08 |
| Total aldehydes, mg/L | 24.46 | 28.91 | 37.81 | 40.03 | 44.48 | 46.70 | 31.14 | 35.58 | 37.81 |
| pH | 3.24 | 3.16 | 3.19 | 3.17 | 3.16 | 3.16 | 3.15 | 3.12 | 3.15 |
| Tasting score | 82.62 | 80.37 | 83.37 | 82.75 | 87.12 | 78.87 | 79.62 | 81.37 | 86.75 |
| | *Saccharomyces cerevisiae* 8-11 | | | | | | | | |
| Alcohol, vol. % | 12.74 | 12.79 | 12.80 | 12.72 | 12.74 | 12.87 | 12.78 | 12.83 | 12.87 |
| Sugar, g/L | 1.74 | 2.01 | 1.48 | 1.48 | 1.10 | 1.04 | 1.67 | 1.00 | 1.17 |
| Total aldehydes, mg/L | 22.24 | 28.91 | 31.14 | 28.91 | 31.14 | 31.14 | 44.48 | 48.93 | 53.38 |
| pH | 3.20 | 3.15 | 3.21 | 3.18 | 3.20 | 3.21 | 3.17 | 3.18 | 3.16 |
| Tasting score | 83.44 | 83.22 | 83.33 | 87.44 | 83.89 | 85.44 | 82.11 | 86.78 | 84.00 |

The established tendencies were also confirmed by the results of the gas chromatographic analysis (Fig. 4, 5, 6). From the group of carbonyl compounds in the obtained wines, acetaldehyde, acetone and 2-butanone had been identified.



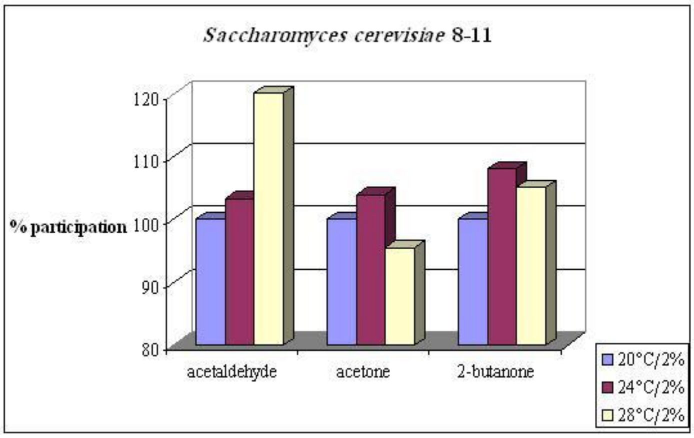
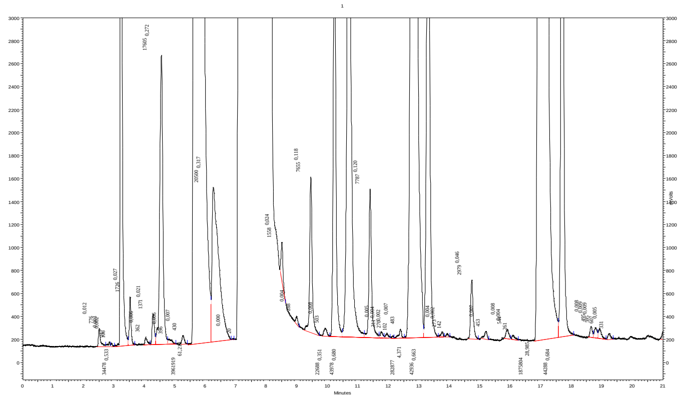
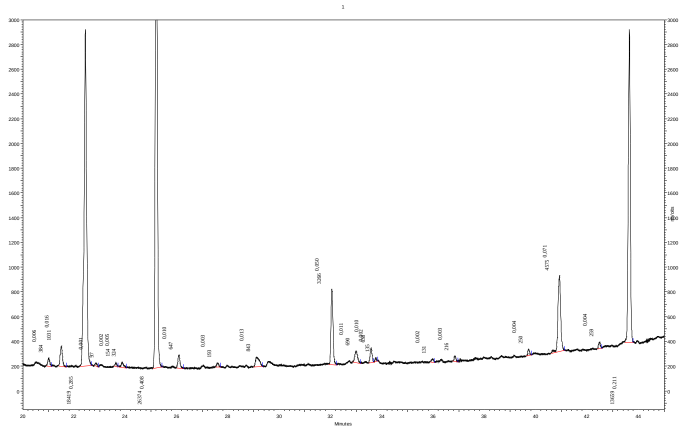




**Figure 4** Identified carbonyl compounds (%) in the experimental wines

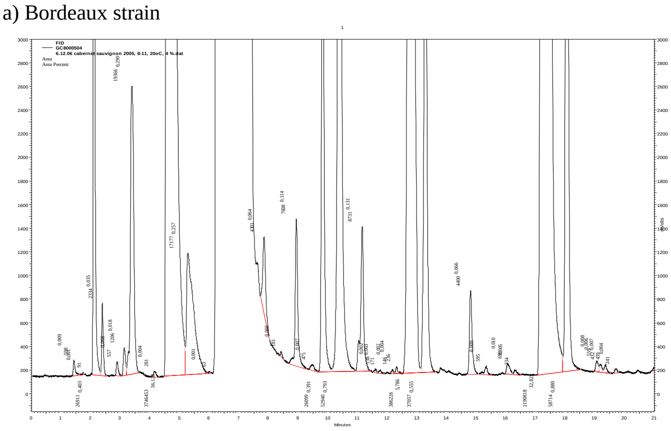


0-21 min



20-45 min

a) Bordeaux strain



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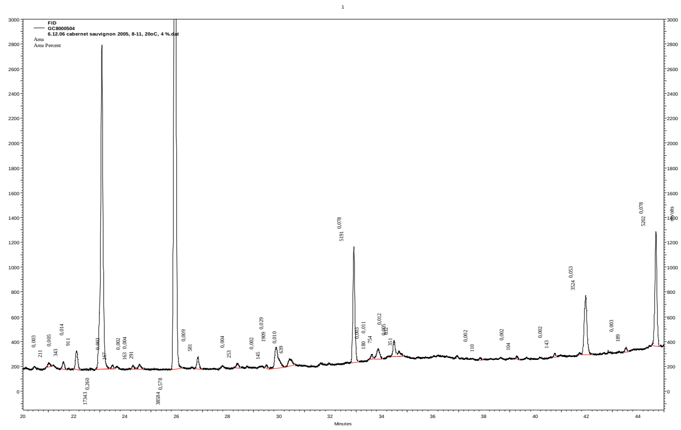


20-45 min

b) 8-11 strain

**Figure 6** Gas chromatographic analysis of the samples, fermented at 20°C/4%, with the studied strains

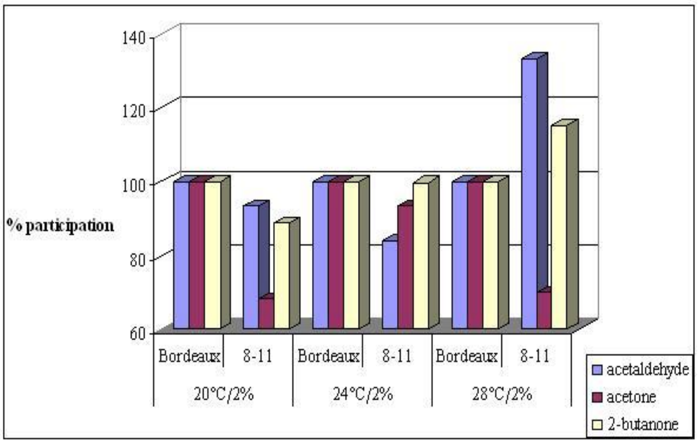
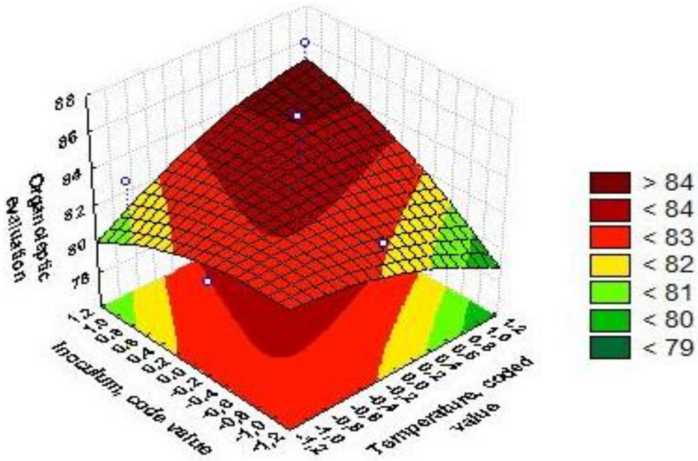
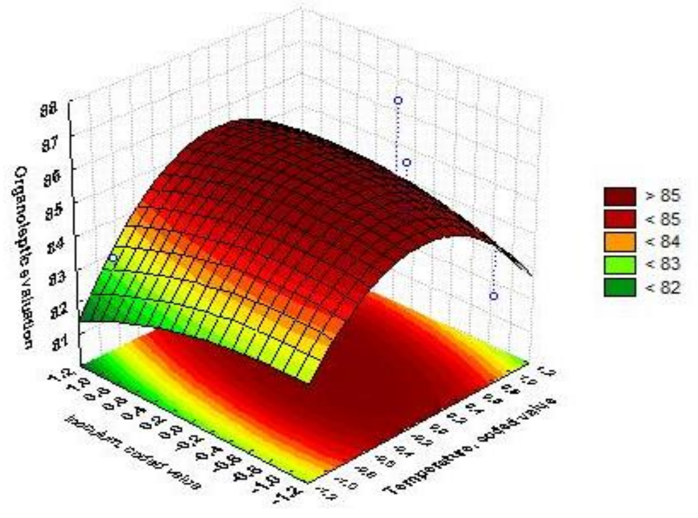



**Figure 5** Distribution of the identified carbonyl compounds in the experimental wines

The presented results demonstrated that under the same conditions the strains showed different ability to produce acetaldehyde, acetone and 2-butanone. Bordeaux strain synthesized the most acetaldehyde at 24ºC, and 8-11– at 28ºC. *Saccharomyces cerevisiae* Bordeaux produced more acetone and 2-butanone at 20°C, and the least acetone at 24°C and 2-butanone at 28°C, respectively. *Saccharomyces cerevisiae* 8-11 strain synthesized the highest quantity of acetone and 2-butanone at 24°C (Fig. 4). During the fermentation at a temperature of 20°C and 24°C, the strain Bordeaux produced more of the studied carbonyl compounds compared to 8-11 strain. At 28°C, 8-11 formed more acetaldehyde and 2-butanone, 33.3% and 15% respectively, and 30% less acetone than Bordeaux (Fig. 5).




a) Bordeaux strain




b) 8-11 strain

**Figure 7** Response surfaces to describe the tasting evaluations of the experimental wines

The results of the mathematical modeling for the influence of the parameters of the fermentation process on the sensory characteristics of the experimental wines confirmed the obtained tasting evaluations (Tab. 1, Fig. 7). The total aldehydes content in the experimental samples did not affect adversely their aromatic and taste qualities. The variant of the strain Bordeaux obtained at 24°C/3% (87.12 points) containing a higher amount of total aldehydes (44.48 mg/L) and the variant of 8-11 strain, obtained at 24°C/2% (87.44 points), containing a lower amount of aldehydes (28.91 mg/L) had the best properties and were evaluated with the highest points.

## CONCLUSION

Under the conditions of the experiment, the studied yeasts had different ability to produce total aldehydes and carbonyl compounds. A more pronounced effect of the temperature than the amount of inoculum yeast culture was observed.

At the beginning of the fermentation, the aldehydes content increased rapidly and reached a peak during the rapid phase (day 5$^{th}$), thereafter their amount began to decrease (day 10$^{th}$, 20$^{th}$).

*Saccharomyces cerevisiae* Bordeaux strain synthesized the most acetaldehyde at a temperature of 24ºC, and *Saccharomyces cerevisiae* 8-11 – at 28ºC.

The stronger effect of the temperature on the fermentation process and the wine components was more pronounced in 8-11 strain than in Bordeaux strain.

During the fermentation at 20°C and 24°C, *Saccharomyces cerevisiae* Bordeaux produced more acetaldehyde, acetone and 2-butanone compared to 8-11. At 28°C, 8-11 strain produced more acetaldehyde and 2-butanone, 33.3% and 15% respectively, and 30% less acetone than Bordeaux.

The total aldehydes content in the experimental samples did not affect adversely their aromatic and taste qualities.

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