Effect of fermentation temperature on oenological parameters and volatile compounds in wine

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Abstract. The increase in temperature caused by climate change is one of the greatest challenges the wine industry has to face. Temperature increase affects sugar and alcohol content, which directly impact the chemical and organoleptic characteristics of wine. This has a serious impact on the competitiveness and profits of companies in the sector. Among the most studied strategies focused on guaranteeing wine quality is the use of yeast strains that are better adapted to the conditions generated by climate change. Therefore, this study seeks to evaluate whether the *Saccharomyces cerevisiae* strains LALVIN CY3079 and UVAFERM WAM maintain their organoleptic characteristics at different temperatures. For this purpose, 3 experimental fermentations were carried out at 16, 20, and 27ºC, respectively. Alcoholic fermentation was monitored (pH, sugars, and microbial population) and general oenological parameters (acetic, citric, malic, succinic, lactic, amine nitrogen, ammonium, and glycerol) were evaluated at the beginning and end of fermentation. In addition, the ethanol content and volatile compounds formed at the end of fermentation were analysed. As a result of these experimental fermentations, it was observed that most of the basic oenological parameters and volatile compounds are modified as a function of fermentation temperature.

1 Introduction

Adapting to new environmental conditions is one of the greatest challenges facing the wine industry [1–4]. Among the climatic factors affecting wine production, temperature rise seems to be the most important [5]. The main effects of climate change in vineyard are an increase in the incidence of plant diseases, variations in sugar and alcohol content that directly affect the chemical and organoleptic characteristics of the wine, leaching and soil erosion [6]. These effects generate a serious impact on the competitiveness and profits of the companies in the sector [7].

Various proposals have been put forward to meet these challenges such as reverse osmosis or the genetic modification of yeasts [1]. However, these strategies have limitations such as significant changes in the microbial ecology of musts and wines that increase the risk of spoilage and organoleptic degradation. Nowadays, there is still not enough information available to determine strategies to obtain quality wine fermented at elevated temperatures using *Saccharomyces cerevisiae*.

Among the most studied strategies to guarantee wine quality is the use of yeast strains that are better adapted to the conditions generated by climate change [8]. In fact, one of the major objectives of the alcoholic beverages industry is to obtain yeast strains that have great potential to carry out alcoholic fermentation and have a favorable impact on wine sensory quality [9]. Therefore, the selection of specialized strains to improve this process has been promoted.

Thanks to the knowledge of the genetics and physiology of *S. cerevisiae*, it has been possible to generate strains with improved fermentation performance and improved organoleptic characteristics such as *S. cerevisiae* LALVIN CY3079 and UVAFERM WAM [10].

Therefore, the aim of this study was to evaluate the impact of temperature on the quality of wine fermented with *S. cerevisiae* using two strains (LALVIN CY3079 and UVAFERM WAM).

2 Materials and methods

2.1 Microorganisms and inocula

The yeast strains used were *S. cerevisiae* LALVIN CY3079 and UVAFERM WAM, both from Lallemand Inc. (Montréal, Canada). Yeasts were maintained on YPD plates (2% glucose, 2% bacto-peptone, 1% yeast extract, 2% agar, w/v) stored at 4°C. To obtain the inocula, a colony was picked from the plates and grown in liquid media at 28°C for 24 h.

2.2 Experimental fermentations

Fermentations were performed in 500 mL flasks containing 400 mL of sterile must, prepared using white grape concentrated must (65.4° Brix; Mostos Españoles S.A., Tomelloso, Spain) and sterile MilliQ purified water to obtain a sugar concentration of 200 ± 10 g/L This must was inoculated with the selected strains of *S. cerevisiae* (10^6 cells/mL) and incubated at the different studied temperatures (16ºC, 20ºC, and 27ºC). All fermentations

were carried out in triplicate. Samples were taken every 24 h to monitor the evolution of sugar consumption, yeast population and pH. Yeast populations were monitored by plating the appropriate dilution in YPD agar and incubated at 28ºC for 48 h. The end of fermentation was reached when the sugar concentration was below 1 g/L. Once this concentration was reached, to eliminate yeasts, the wine was filtered using a sterile funnel and 0.45 µm pore filters (MF-MilliporeTM, Ref.: HAWP04700).

2.3 Wine characterization

After filtration, different chemical compounds were determined using a UV-3600 UV-Vis-NIR Spectrophotometer (Shimadzu Scientific Instruments, Inc., MD, USA): glucose and fructose, L-malic acid, L-lactic acid, L-acetic acid, glycerol, citric acid, total and free sulfites (Biosystems, Barcelona Spain) and succinic acid (NEOGEN Europe Ltd., Scotland, UK) and ethanol (R-Biopharm AG., Darmstadt, Germany).

2.4 Analysis of volatile compounds in Cava and lees

The extraction of volatile compounds in wine was performed by Head-Space Solid Phase Microextraction (HS-SPME). It was carried out using a 2 cm long Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/ CAR/PDMS) fiber supplied by Supelco (Bellefonte, PA, USA). Samples of 5 mL were placed in 10 mL vials. After 15 min of equilibration at 60°C under continuous agitation (250 rpm), the fiber was exposed to the headspace for 40 min.

Chromatographic analysis was carried out in a 6890N Network GC system (Agilent, Palo Alto, CA, USA) coupled to MS Agilent technologies 5973 Network selective detector (Agilent, Palo Alto, CA, USA). Helium was used as a carrier gas. Separations were accomplished in a DB Wax USN 125-7031 column (60 m × 0.25 mm × 0.25 µm) (Agilent, Palo Alto, CA, USA). A splitless injector suitable for SPME was used. After extraction, the fibre was removed from the headspace vial and inserted directly into the injection port of the GC. The SPME fibre was thermally desorbed for 5 min at 260°C.

The initial temperature of the column was 40°C for 10 min, and this was subsequently increased at 4°C/min up to 75°C, then temperature was increased at 2°C/min up to 260°C and hold for 5 min using splitless injection mode. GC-MS detection was performed in complete scanning mode (SCAN) in the 40–350 amu mass range with two scans per second. Electron impact mass spectra were recorded at an ionization voltage of 70 eV and ion source of 280°C. Volatiles concentrations reported were calculated by dividing the peak area of the compounds of interest by the total area, obtaining the relative abundance of each compound. The relative response factor was considered to be 1. Identification was performed by comparison of their mass spectra with those of the mass spectra library database Wiley 6.0., and their retention times with those of pure standards when they were available.

2.5 Statistical analysis

All assays were performed in triplicate and in a randomized run order. The statistical analysis was performed using RStudio version 1.2.5033. The results are reported as the means ± standard error (SE) for parametric data. A two-way ANOVA and the corresponding pairwise post-hoc comparison of the means were conducted using Bonferroni's correction, with significance level of 0.05. We carefully checked, with ad hoc inference tests, that the assumptions of ANOVA were fulfilled, that is, that our data were independent, normally distributed for each factor and with equal variances. In case that one of these hypotheses failed, we implemented suitable non-parametric tests alternative but equivalent to ANOVA. Principal component analysis (PCA) was also carried out to determine differences between experimental wines.

3 Results and Discussion

3.1 Kinetics of Alcoholic Fermentation (AF)

In this study, three different temperatures of fermentation (16ºC, 20ºC, and 27ºC) were analyzed using two strains of *S. cerevisiae* (LALVIN CY3079 and UVAFERM WARM). At higher temperatures there was a faster consumption of sugars compared to lower temperatures (Table 1).

Table 1. AF speed of LALVIN CY3079 and UVAFERM WAM strains at 16ºC, 20ºC, and 27ºC.

Yeast Strain	Temperature (ºC)	Peak population day	Maximum population level reached (cfu ml^{-1})	Duration of fermentation (days)	Maximum fermentation rate (g l^{-1} days^{-1})	AF speed (g·L^{-1} ·day^{-1})
LALVIN	16ºC	6	1,0E+08	7	63,72	31 ± 2,83
	20ºC	4	1,0E+08	6	69,34	47 ± 7,07
	27ºC	2	9,30E+07	5	121,21	64 ± 10,01
UVAFERM	16ºC	6	5,0E+07	7	104,25	34 ± 2,93
	20ºC	1	5,30E+07	5	66,94	46 ± 5,74
	27ºC	2	9,30E+08	5	99,33	55 ± 8,66

Results are expressed as mean ± standard deviation of triplicates. AF: Alcoholic fermentation.

This difference was more significant in the fermentation at 16ºC compared to the other two since it took more days for yeasts to consume sugar. This kinetics agrees with previous studies [11-13].

As for the differences between strains in sugar consumption, their behavior was similar in all fermentations since the total duration of all fermentations was the same (Fig. 1). However, on an overall level, the UVAFERM WAM strain had a slightly higher fermentation rate in the early stages of alcoholic fermentation while on average the strain LALVIN CY3079 strain is faster in most of the fermentations.

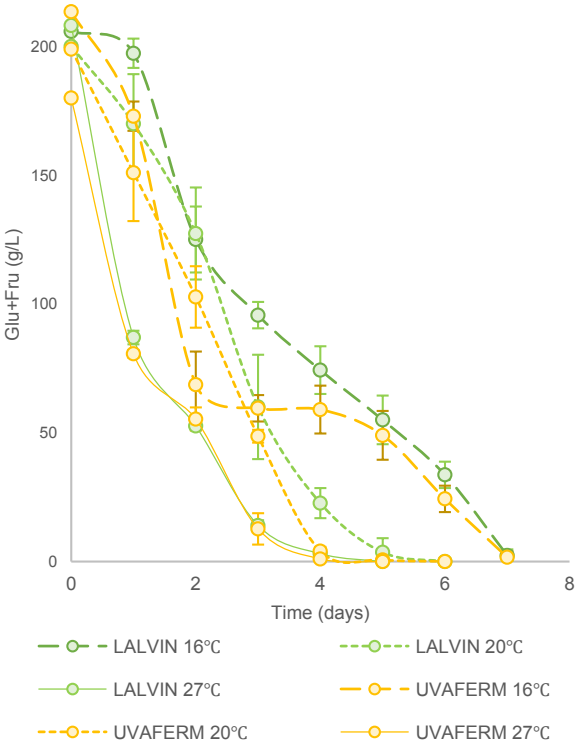

Figure 1. Evolution of AF through sugar consumption by yeasts.

3.2 Effect of temperature on yeast growth

The fermentation at 16ºC started more slowly (Fig. 1), as it can be seen by its longer exponential phase, while the rest of the fermentations were characterized by a short exponential phase and reach the lag phase more quickly. In fact, the fermentation at 16°C takes longer to reach the maximum population (1.0E+08 cfu ml^{-1}), being the sixth day of fermentation (Table 1). In contrast, both the 20°C and 27°C fermentations reach this maximum on the second day and maintain the stationary phase until the end of fermentation. This stationary phase is prolonged in fermentations at 20ºC and 27ºC while at 16ºC it is reached right at the end of fermentation.

Thus, as expected from the scientific literature, the rate of yeast growth and alcoholic fermentation increases with increasing temperature, with maximum rates generally occurring at temperatures between 20 and 25°C. In addition, the higher the temperature, the shorter the fermentation time. [13, 14].

In addition, in all fermentations, there is no latency phase in the growth of microorganisms. According to Jackson [15], this is due to the use of active dry yeast as inoculum, since it has been seen that in the wineries that use these inocula, the yeasts, being pre-adapted, show an apparent lack of latency.

3.3 Changes in wine composition

As expected, the temperature had an impact on wine composition and most of the chemical compounds studied showed significant differences at the different fermentation temperatures (Table 2).

Regarding the compounds in which there are significant differences, we see that in the case of total sulfites, their amount increases the lower the fermentation temperature. This has been found in some studies [16], although the production of sulfites has been studied more extensively in relation to the type of strain.

Table 2. Characterisation of initial must and wines fermented at 16ºC, 20ºC, and 27ºC with UVAFERM WAM and LALVIN CY3078 strains.

	Sugar (g/L)	L-malic acid	Citric acid (g/L)	α-NH2 (mg/L)	Ammonium (mg/L)	pH
Must	201±11,63	1,22 ±0,50	0,16 ± 0,03	87,38 ± 12,60	294± 0,03	3,66 ± 0,07

	L-malic acid (g/L)	Citric acid (g/L)	α-NH2 (mg/L)	Ammonium (mg/L)	pH	Ethanol (% (v/v))
UVAFERM 16ºC	1,56 ± 0,32^{a}	0,20 ± 0,00^{a}	23,21 ± 3,69^{a}	3,12 ± 0,19^{a}	3,42 ± 0,9^{a}	11,56 ± 0,18^{a}
UVAFERM 20ºC	1,54 ± 0,21^{a}	0,22 ± 0,04^{a}	22,85 ± 3,48^{b}	4,13± 0,05^{a}	3,31 ± 0,04^{a}	12,03 ± 0,17^{a}
UVAFERM 27ºC	1,39 ± 0,40^{a}	0,19 ± 0,04^{a}	13,75 ± 3,79^{c}	0,26 ± 0,17^{b}	3,51 ± 0,03^{a}	12,94 ± 0,45^{a}
LALVIN 16ºC	1,64 ± 0,18^{a}	0,40 ± 0,07^{a}	23,01 ± 1,48^{a}	2,91± 0,16^{a}	3,43 ± 0,08^{a}	11,51 ± 0,09^{a}
LALVIN 20ºC	1,50 ± 0,06^{a}	0,20 ± 0,07^{b}	13,12 ± 2,77^{c}	3,83 ± 1,97^{a}	3,40 ± 0,12^{a}	12,03 ± 0,17^{a}
LALVIN 27ºC	1,35 ± 0,25^{a}	0,31 ± 0,07^{ab}	12,73 ± 1,36^{c}	0,70 ± 0,12^{b}	3,47 ± 0,01^{a}	14,82 ± 3,01^{a}

	Succinic acid (mg/L)	Acetic acid (g/L)	Glycerol (g/L)	Total sulfite (mg/L)	Free sulfite (mg/L)
UVAFERM 16ºC	0,28 ± 0,01^{a}	0,66 ± 0,20^{a}	8,96 ± 0,06^{a}	58,00 ± 3,38^{ab}	4,99 ± 0,51^{a}
UVAFERM 20ºC	0,34 ± 0,01^{a}	1,56 ± 0,41^{b}	8,96 ± 0,97^{a}	53,58 ± 8,08^{ab}	4,78 ± 1,08^{a}
UVAFERM 27ºC	0,34 ± 0,00^{a}	0,34 ± 0,17^{c}	9,99 ± 0,83^{a}	54,44 ± 1,95^{c}	8,96 ± 1,31^{b}
LALVIN 16ºC	0,39 ± 0,00^{a}	0,48 ± 0,15^{a}	9,61 ± 0,09^{a}	45,88 ± 4,53^{ab}	4,57 ± 0,18^{a}
LALVIN 20ºC	0,37 ± 0,00^{a}	0,84 ± 0,22^{b}	9,78 ± 0,21^{a}	52,76 ± 1,62^{ab}	1,61 ± 1,34^{c}
LALVIN 27ºC	0,44 ± 0,00^{a}	0,34 ± 0,17^{c}	9,90 ± 0,07^{a}	42,51 ± 1,52^{c}	6,46 ± 0,40^{d}

Different letters denote statistically significant differences between temperature according to a Bonferroni post-hoc comparison test.

In the case of amine nitrogen, it can be established that the higher the temperature, the lower the amount of amine nitrogen in the wine. This may be because the higher the temperature, the greater the amount of succinic acid generated, and nitrogen has a negative effect on the production of this compound [17]. Both amine nitrogen and ammonium are sources of nitrogen used by yeasts for growth, so the high growth of the microbial population in fermentations means that the amount of these compounds is lower.

In relation to the amount of acetic acid, it has been found that the maximum amount is reached at 20ºC while the lowest concentration occurs at 27ºC. This is contrary to what is established in several studies in which it is established that the higher the temperature, the greater the amount of this compound [11, 18]. These differences could be caused by the fact that its production is influenced by the phytosterol content. When there are high amounts of phytosterols, there is an increase in the production of succinic acid and a decrease in acetic acid. In addition, these compounds are stable at elevated temperatures [17].

As for citric acid, it has been observed that in the experimental fermentations with both strains it reaches its peak at 20ºC. Regarding this organic acid, there are no in-depth studies on its relationship with AF temperature, and in some cases, it has even been found that its kinetics do not vary significantly as a function of temperature or pH [18].

The compounds that remained stable during fermentations at different temperatures were malic acid, succinic acid, glycerol, ethanol and pH. With respect to malic acid, it remained stable since it has been observed that it maintains similar values at temperatures of 16ºC and 24ºC [19], while a slight increase has been observed at temperatures of 30ºC [18]. In addition, no changes in malic acid are evident since malolactic fermentation has not taken place.

As for ethanol and glycerol, although some studies have shown temperature-dependent changes [19, 20], it has also been shown that their stability to temperature changes depends on the yeast strain, since they have different optimum temperatures for their production [21]. Although there are no significant differences in ethanol, there is a tendency for the alcohol content to increase as the temperature increases. In addition, ethanol was more influenced by pH than by temperature [18].

Another factor that conditions AF and is not significantly affected by temperature is pH [16]. Although temperature is a factor to be taken into account in relation to pH, many authors consider that this parameter is more affected by ethanol production [18,22].

Furthermore, the results obtained were subjected to a Principal Component Analysis (Fig. 2) to group the experimental wines and to see the similarities and differences after AF. First two components explain 57.07% of the variance of the wines.

Principal component 1 (PC1), explains 40.20% of the variance and the variables showing a positive correlation are acetic acid, amine nitrogen, ammonia, total sulfites and citric acid, while malic and succinic acid, fermentation speed, ethanol, glycerol, free sulfites, and pH showed a negative correlation.

Principal component 2 (PC2) explains 16.85% of the variance. In this component the compounds that showed a positive correlation were ammonia, acetic, malic and succinic acid, ethanol, glycerol and fermentation rate. In contrast, amine nitrogen, sulfites, citric acid and pH showed a negative correlation.

Furthermore, this PCA separates the samples according to the fermentation temperature, which indicates that there is a difference in the composition of the wines according to the fermentation temperature and especially of the fermentations at 27ºC with respect to the rest (16ºC and 20ºC). At that temperature in turn shows a difference between the strains. In particular, more variability was observed in fermentation with strain LALVIN CY3079.

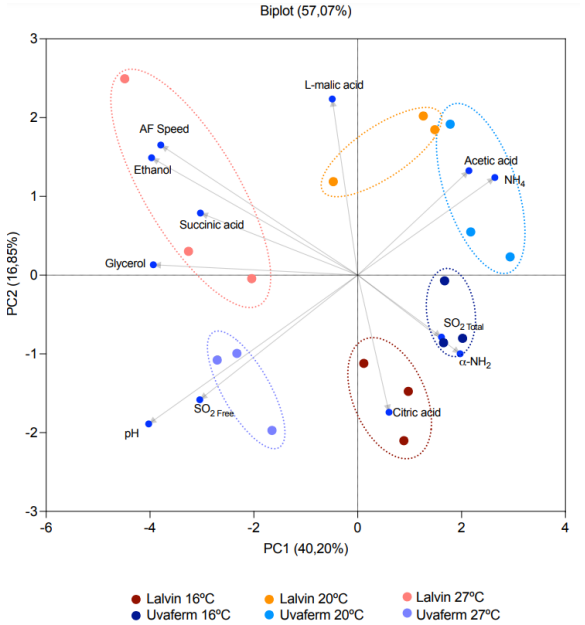

Figure 2. Principal component analysis (PCA) biplot of experimental wines.

3.4 Changes in the volatile compounds

Table 3 shows the relative abundance of volatile compounds identified in the experimental wines. Esters were the major volatile compounds, highlighting ethyl decanoate and ethyl octanoate, contributing to aroma with fruity notes. It has also been shown that most of these compounds are significantly affected by temperature, as seen in previous studies [19, 23].

As for how temperature particularly affects each of the compounds, we see that there are some volatiles whose concentration increases proportionally with temperature, such as isoamyl acetate and phenylethyl acetate. In contrast, there are others such as ethyl hexanoate and ethyl dodecanoate whose amount is inversely proportional to temperature [24].

However, there are several compounds for which there is still no consensus and show different results, such as ethyl acetate. Some studies determine that in fermentations at lower temperatures its proportion increases [25], while others show it to be directly proportional to temperature [17]. In fact, in the case of this study it is observed that it reaches higher concentrations at extreme temperatures (16ºC and 27ºC) while it decreases at intermediate temperatures (20ºC).

Regarding some of the major volatile compounds in wines, ethyl hexanoate, like ethyl octanoate and ethyl decanoate significant differences among the samples were found but not among the different strains. The contents of ethyl hexanoate, ethyl octanoate and ethyl decanoate are related to lipid metabolic pathways and were found to increase with the increase of the corresponding fatty acids [26].

Table 3. Relative abundance of volatile compounds identified in wines fermented at 16ºC, 20ºC, and 27ºC.

		LALVIN			UVAFERM		
Núm. CAS	Aroma	16º	20º	27º	16º	20º	27º
141-78-6	ethyl ethanoate	1.56 ± 0.04^{a}	1.26 ± 0.15^{a}	2.06 ± 0.07^{b}	1.71 ± 0.07^{a}	1.34 ± 0.04^{a}	2.34 ± 0.52^{b}
123-66-0	**ethyl hexanoate**	6.21 ± 0.39^{a}	6.67 ± 1.07^{a}	5.31 ± 0.17^{b}	5.93 ± 0.88^{a}	7.3 ± 0.18^{a}	4.8 ± 0.67^{b}
106-32-1	**ethyl octanoate**	32.32 ± 0.57^{a}	37.36 ± 0.11^{b}	23.35 ± 0.09^{c}	31.48 ± 0.94^{a}	36.98 ± 0.44^{b}	24.58 ± 1.41^{c}
110-38-3	**ethyl decanoate**	18.08 ± 0.32^{a}	16.11 ± 2.18^{a}	11.91 ± 0.43^{b}	17.09 ± 0.63^{a}	14.2 ± 0.53^{a}	11.82 ± 1.36^{b}
103-45-7	phenethyl acetate	1.14 ± 0.17^{a}	0.81 ± 0.05^{a}	1.14 ± 0.08^{b}	1.23 ± 0.07^{a}	0.94 ± 0.13^{a}	1.19 ± 0.17^{b}
106-33-2	ethyl dodecanoate	2.58 ± 0.21^{a}	1.48 ± 0.17^{b}	0.75 ± 0.09^{c}	2.64 ± 0.27^{a}	1.39 ± 0.04^{b}	0.62 ± 0.48^{c}
060-12-8	**phenethyl alcohol**	2.57 ± 0.09^{a}	2.49 ± 0.09^{b}	6.69 ± 0.35^{c}	2.88 ± 0.06^{a}	2.61 ± 0.26^{b}	6.69 ± 0.58^{c}
628-97-7	ethyl hexadecanoate	0.89 ± 0.29^{a}	0.61 ± 0.15^{a}	0.7 ± 0.43^{a}	0.88 ± 0.12^{a}	0.67 ± 0.22^{a}	0.48 ± 0.23^{a}
334-48-5	decanoic acid	1.51 ± 0.29^{a}	1.24 ± 0.27^{a}	0.89 ± 0.60^{a}	1.54 ± 0.27^{a}	1.18 ± 0.17^{a}	1.15 ± 0.24^{a}
123-92-2	**isoamyl acetate**	5.38 ± 0.07^{a}	4.35 ± 0.90^{b}	5.17 ± 0.26^{a}	6.77 ± 0.01^{c}	4.68 ± 0.21^{d}	7.51 ± 0.01^{c}
123-51-3	**isoamyl alcohol**	2.82 ± 0.01^{a}	2.9 ± 0.40^{a}	8.94 ± 0.20^{b}	3.52 ± 0.2^{a}	3.01 ± 0.11^{a}	8.34 ± 0.00^{b}
124-06-1	ethyl tetradecanoate	0.27 ± 0.06^{a}	0.16 ± 0.03^{a}	0.22 ± 0.31^{a}	0.3 ± 0.03^{a}	0.18 ± 0.07^{a}	0.17 ± 0.01^{a}

Different letters denote statistically significant differences according to a Bonferroni post-hoc comparison test. Major compounds which showed significant differences between temperatures are in bold.

Finally, it is worth mentioning that both yeast strains presented a similar volatile profile. In general, volatiles such as ethyl acetate, ethyl 9-decenoate or isoamyl acetate related to sweet and fruity aromas and compounds such as phenylethyl acetate and phenylethyl alcohol that provide the floral aroma are present in both strains [23]. This aromatic profile is consistent with the type of aromas usually found in white wines [27].

4 Conclusions

This study shows that the microbial growth varies significantly according to the different fermentation temperatures studied. As expected, the higher the temperature, the higher the fermentation rate.

Regarding the differences in the composition and organoleptic properties of wines fermented at temperatures of 16ºC, 20ºC, and 27ºC, it has been shown that temperature significantly affects the parameters of amine nitrogen, citric acid, acetic acid and total sulfites.

In addition, significant differences in the relative abundance of volatile compounds were also shown. Most of these differences occurred when the fermentation temperature was increased to 27ºC. Also, it is worth mentioning that the strain affects the synthesis of some compounds but plays a minor role.

Therefore, it can be seen how the increase in temperature because of climate change will result in a change in the composition and organoleptic characteristics of wine. This increase in temperature can generate wines with lower acidity and nitrogen compounds, higher sulfite content and a tendency to increase alcohol content. In addition, some ethyl esters responsible for the fruity aroma are reduced and the volatiles of the ethanol family increase.

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