SO$_2$ protects the amino nitrogen metabolism of *Saccharomyces cerevisiae* under thermal stress

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Summary
Thermal stress conditions during alcoholic fermentation modify yeasts’ plasma membrane since they become more hyperfluid, which results in a loss of bilayer integrity. In this study, the influence of elevated temperatures on nitrogen metabolism of a *Saccharomyces cerevisiae* strain was studied, as well as the effect of different concentrations of SO$_2$ on nitrogen metabolism under thermal stress conditions. The results obtained revealed that amino nitrogen consumption was lower in the fermentation sample subjected to thermal stress than in the control, and differences in amino acid consumption preferences were also detected, especially at the beginning of the fermentation. Under thermal stress conditions, among the three doses of SO$_2$ studied (0, 35, 70 mg l$^{-1}$ SO$_2$), the highest dose was observed to favour amino acid utilization during the fermentative process, whereas sugar consumption presented higher rates at medium doses.

Introduction
Changes in environmental conditions such as temperature, aeration and nutrients can significantly affect yeast performance during alcoholic fermentation. The application of high temperatures during alcoholic fermentation is useful in order to extract the greatest quantity of tannins and polyphenols in red wine making and to obtain wines with low alcohol content, in accordance with current consumer tastes. However, yeasts cannot regulate their internal temperature and thermal stress causes cellular damage, leading to adverse effects on yeast cell physiology. Thermal stress produces an increased fluidity and membrane permeability to protons and other ions (Bischof et al., 1995; Piper et al., 1997), denaturation of membrane proteins (Lepock et al., 1993), membrane blebbing (Martínez de Marañón et al., 1999) or cell lysis (Gershfeld and Murayama, 1988). Hazel (1995) and Martínez de Marañón and colleagues (1999) found that when temperature exceeds the physiological range, membranes become hyperfluid and destabilization of the lamellar phase occurs, which results in a loss of bilayer integrity. Moreover, yeast metabolism mainly depends on the uptake of nutrients driven by permeases and amino acid transport in yeast is also influenced by fatty acid composition (Ayestarán et al., 1995; 1998) and membrane fluidity (Mishra and Prasad, 1989). Ethanol concentration also affects the composition, structure and permeability of the plasma membrane, and consequently, the activity of glucose, ammonium and amino acids transport systems is altered (Leão and van Uden, 1982; 1983). Furthermore, ethanol accelerates the passive entrance of protons from the medium to the cytoplasmic matrix (Ingram et al., 1986), and dissipates the proton motive force necessary for amino acid transport into the cell (Leão and van Uden, 1984; Cartwright et al., 1986). Guerzoni and colleagues (1999) found that, at fermentation temperatures above 30°C, ethanol toxic action towards yeasts is enhanced.

SO$_2$ has been used as a preservative agent because of its several functions in wine conservation. *Saccharomyces cerevisiae* is relatively resistant to sulphite. The main protein involved in sulphite resistance in this yeast is the sulphite pump Ssulp (Avram and Bakalinsky, 1997; Park and Bakalinsky, 2000) that mediates sulphite efflux. On the other hand, SO$_2$ could influence the utilization of amino acids by yeast although it is not clear whether it negatively alters nitrogen metabolism or whether SO$_2$ is a protective agent for yeasts against stress conditions during fermentation. Maier and colleagues (1986) found that SO$_2$ inside the cell would induce changes in enzymatic 3D-conformations and would cause depletion in the yeast’s cellular ATP content due to its effects on glycolysis and respiratory chain phosphorylation. Caridi (2002; 2003) suggested that it is possible that SO$_2$, inositol and catechin act as protectors of *S. cerevisiae*, minimizing the adverse effects produced in this yeast under stress conditions, thus improving wine quality. His results showed significant correlations between the addition of these protectants and the change in metabolic behaviour of yeasts.
under concomitant thermal and osmotic stress. He suggested that some strains increased fermentation vigour and produced more normal metabolite profiles. However, whether those conditions stimulated nitrogen accumulation was not reported.

For these reasons, the aims of this work were: (i) to study the influence of high temperatures on nitrogen metabolism of a \textit{S. cerevisiae} strain and (ii) to observe the effect of different concentrations of SO$_2$ on the nitrogen metabolism of this yeast when subjected to heat stress.

\textbf{Results and discussion}

\textit{Fermentation kinetics and general parameters}

Sugar consumption (%) of control fermentation is compared with that of thermal stress fermentation (F35) in Fig. 1A; these two fermentations were performed with the same initial level of SO$_2$ (35 mg l$^{-1}$). In this figure, it is observed that the initial sugar consumption rate was similar in both cases; however, after 4 days, the control sample presented a higher rate than the one subjected to thermal stress. In the control sample, 99\% of must sugar was consumed, whereas in the sample subjected to thermal stress yeasts consumed 90\% of sugar. In cells subjected to thermal stress the passive proton influx towards the cytoplasm is increased, so the intracellular pH decline and this can be a major factor contributing to the inhibition of fermentation rate (Neves and François, 1992). Besides, at the plasma membrane, the increase of the passive proton influx due to thermal stress will act to dissipate the electrochemical potential gradient maintained across this membrane by the action of plasma membrane H$^+\text{-ATPase}$ (Serrano, 1991). The electrochemical potential gradient is essential for vital functions such as the maintenance of potassium balance and the regulation of intracellular pH.

\textbf{Fig. 1.} Fermentation kinetics (A) at two different temperatures (29$^\circ$C and 35$^\circ$C) in the presence of 35 mg l$^{-1}$ of SO$_2$ (B) thermal stress (35$^\circ$C) in the presence of 0, 35, 70 mg l$^{-1}$ of SO$_2$. 

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In Table 1 general parameters of the wines are presented. It may be observed that volatile acidity was greater in the wines obtained at high temperatures than in the control sample. This can be attributed to the lower fermentation rate of thermal stress fermentation. Volatile acidity of wines was inferior in all cases to the threshold found by Peynaud (1993) as undesirable for wine aroma (0.6 g HAc l⁻¹). Wines coming from fermentations subjected to thermal stress presented lower ethanol content than the control wine because there was an unfermented sugar residue. Caridi and colleagues (1999) also found that yeasts subjected to stress modified their metabolic behaviour and, probably as a defence mechanism, gave low ethanol yield and abnormal high acetic acid production. F35 sample showed a pH value slightly higher than the other samples. pH values in all wines were appropriate to their later stabilization and conservation, and they were in all cases between the range (3.0–3.67) that Amerine and Ough (1976) found as optimal for wine conservation. Total acidity, as occurs with pH, was slightly lower in F35 sample than in the other samples. In all wines, total acidity values were optimal for wine conservation. Glycerol content in F70 sample was lower than in the other samples. It seems that the higher dose of SO₂ (70 mg l⁻¹) acts as a protectant for yeasts.

Utilization of amino nitrogen during fermentation

Amino nitrogen, which represents the most important nitrogen fraction of the assimilable nitrogen by yeasts during the growing phase, was consumed both in the control sample and in F35 sample, mainly at the beginning of the fermentation (Table 2), before the appearance of appreciable quantities of ethanol, which difficult the nutrient transport inside the cell (O’Connor-Cox and Ingle-dew, 1989). However, comparing both fermentations, it can be observed that, at the beginning of fermentation, amino nitrogen consumption was greater (P < 0.05) in the control sample than in the F35 sample. Llauradó and colleagues (2005) also observed that yeast metabolism is affected by fermentation temperature. Previous physiological studies (Pizarro et al., 2008) have revealed that protein translation rates, cell membrane fluidity, RNA secondary structure stability, enzymatic activity, protein

### Table 1. Enological parameters of wines.

|                   | Control (29°C, 35 mg l⁻¹ SO₂) | F35 (35°C, 35 mg l⁻¹ SO₂) | F0 (35°C, 0 mg l⁻¹ SO₂) | F70 (35°C, 70 mg l⁻¹ SO₂) |
|-------------------|-------------------------------|--------------------------|------------------------|--------------------------|
| pH                | 3.09 ± 0.01                   | 3.20 ± 0.01              | 3.12 ± 0.03            | 3.11 ± 0.02              |
| Alcohol content (% v/v) | 10.91 ± 0.02                 | 10.28 ± 0.08             | 9.38 ± 0.01            | 9.24 ± 0.12              |
| Total acidity (g l⁻¹) | 7.4 ± 0.0                     | 6.7 ± 0.2                | 7.5 ± 0.3              | 7.3 ± 0.1                |
| Volatile acidity (g l⁻¹) | 0.33 ± 0.02                   | 0.46 ± 0.05              | 0.41 ± 0.01            | 0.42 ± 0.01              |
| Reducing sugar (g l⁻¹) | 1.6 ± 0.4                     | 20.5 ± 4.3               | 35.9 ± 3.2             | 42.5 ± 0.4               |
| Glycerol (g l⁻¹) | 7.8 ± 0.1                     | 7.1 ± 0.1                | 6.9 ± 0.1              | 6.4 ± 0.1                |

All parameters listed with standard deviation.

a. Expressed as tartaric acid.

b. Expressed as acetic acid.

25% reducing sugar  | 50% reducing sugar  | Wine
|-------------------|-------------------|-------------------|
| Control           | 93 ± 3 a          | 27 ± 1 a          | 7 ± 2 a              |
| F35               | 80 ± 2 b          | 22 ± 2 a          | 10 ± 1 b             |
| F0                | 76 ± 11 b         | 51 ± 7 b          | 6 ± 1 ac             |
| F70               | 94 ± 4 a          | 45 ± 6 b          | 4 ± 1 c              |

Means within the same column followed by different letters are significantly different (P < 0.05).
The increased level of ROS damages proteins and cell environment under stress conditions, such as thermal stress. Balance is disturbed when cells are exposed to diverse environmental conditions. The control of ROS (Reactive Oxygen Species) keeps balance normal physiological conditions, degeneration and scavenging of ROS (Novo et al., 2004). During the second half of fermentation (from 50% consumed sugar to wine), there were negligible levels of amino nitrogen consumption because high ethanol concentrations affected the composition and permeability of the plasma membrane and thus the amino acid transport systems were also altered.

Comparing the three fermentations subjected to heat stress with different SO2 concentrations (F0, F35, F70), it may be observed that, at the beginning, greater levels of amino nitrogen were consumed in F70 sample than in F0 and F35 samples, whose consumption was similar (Table 2). Therefore, high concentrations of SO2 seem to have favoured the amino nitrogen consumption. Under normal physiological conditions, degeneration and scavenging of ROS (Reactive Oxygen Species) keep balance to avoid molecular damage (Herrero et al., 2008). This balance is disturbed when cells are exposed to diverse environmental stress conditions, such as thermal stress. The increased level of ROS damages proteins and cell membranes, and therefore, a number of cellular processes are affected, such as nitrogen compounds transport through the plasma membrane. As SO2 is an antioxidant, it could, at high concentrations (70 mg l\(^{-1}\)), act as a protector against ROS, improving membrane transporters behavior. In the exponential phase of fermentation (25–50% consumed sugars), amino nitrogen consumption was greater (\(P<0.05\)) in the F70 and F0 samples than in F35 sample. Therefore, both temperature and SO2 concentration have an effect on nitrogen metabolism, which may affect the growth rate and amino nitrogen preferences.

**Influence of fermentation temperature on the amino acids utilization**

In both fermentations (control and F35) the greater bulk of amino acids were consumed in the initial stage of fermentation (25% consumed sugar) (Table 3). Throughout all the fermentation, total amino acids consumption was greater in the control sample than in the sample subjected to heat stress. According to the ratio obtained, there was greater consumption of glutamic acid, asparagine, glycine, proline, isoleucine and tryptophan in the control sample than in the sample subjected to heat stress (F35). In this sample, only aspartic acid and alanine were more consumed throughout the whole process. Beltrán and colleagues (2004) found that low-temperature fermentation produced similar metabolic effects to those obtained in nitrogen-limited fermentation. These authors observed

| Table 3. Consumption (mg l\(^{-1}\)) of amino acids in control sample and in F35 sample during fermentation. |
|---------|----------|----------|----------|----------|----------|
|         | 25% consumed sugar | 50% consumed sugar | Wine | Ratio* |
|         | Control | F35 | Control | F35 | Control | F35 | Control/F35 |
| PSER    | 2.1 a   | 2.1 a | -4.2 a | -3.6 a | -7.6 a | -3.9 b | - |
| ASP     | 0.4 a   | 5.8 a | 6.9 a | 1.4 b | -1.9 a | -0.3 b | 0.8 |
| GLU     | 16.3 a  | 15.8 a | 5.3 a | 8.0 b | 5.0 a | -4.7 b | 1.4 |
| PEA     | 1.3 a   | 1.3 a | -1.4 a | -1.3 a | 1.4 a | -0.3 b | - |
| SER     | 8.9 a   | 8.9 a | 0.5 a | 0.5 a | -0.3 a | 0.0 a | 1.0 |
| ASN     | 3.5 a   | 3.4 a | 0.1 a | 0.3 a | 1.2 a | -0.7 b | 1.6 |
| GLY     | 0.1 a   | 2.1 b | 0.3 a | 2.5 a | 0.0 a | -0.2 a | 1.5 |
| GABA    | 14.0 a  | 14.0 a | -1.1 a | -1.5 a | -0.3 a | -0.4 a | 1.0 |
| ALA     | 26.5 a  | 33.0 a | 17.5 a | 11.1 a | -2.0 a | 1.2 a | 0.9 |
| ARG     | 245.7 a | 197.5 b | 10.1 a | 58.1 b | -6.0 a | -2.9 b | 1.0 |
| PRO     | -146.9 a | -143.9 a | 152.4 a | 64.3 b | 49.3 a | 85.8 b | 11.0 |
| VAL     | 8.2 a   | 7.7 b | 1.5 a | 1.9 b | -1.2 a | -1.0 a | 1.0 |
| MET     | 0.4 a   | 2.2 b | 3.6 a | 1.1 b | -0.6 a | -0.5 a | 1.2 |
| ILE     | 7.6 a   | 15.0 a | 1.5 a | 0.3 a | 1.4 a | 0.9 a | 1.5 |
| LEU     | 4.9 a   | 5.0 b | 1.1 a | 0.9 b | -1.6 a | -1.6 a | 1.0 |
| PHE     | 4.4 a   | 3.3 a | 1.7 a | 1.8 a | -1.3 a | -0.3 a | 1.0 |
| TRP     | 12.4 a  | 15.2 b | 5.0 a | 2.1 b | -0.5 a | -9.7 b | 2.2 |
| LYS     | 0.07 a  | 0.85 b | 1.7 a | -0.6 a | 1.4 a | -0.9 a | - |
| Aa consumed (mg l\(^{-1}\)) | 352.8 a | 316.3 b | 208.3 a | 155.7 b | 58.3 a | 86.7 a | 1.2 |
| Amino-N consumed (mg N l\(^{-1}\)) | 75.6 a | 61.3 b | 26.7 a | 30.4 a | 4.9 a | 7.4 a | - |

Means within the same row followed by different letters are significantly different (\(P<0.05\)).

a. As the ratio between total amino acids consumed in control and F35 samples was 1.2, amino acids with a ratio > 1.2 were more consumed in control sample.
that in fermentations that were not nitrogen-limited, yeasts cells consumed much less nitrogen at 13°C than at 25°C; this is due to a decrease in the fluidity of the plasma membrane at low temperatures, which considerably reduces the molecular motion of phospholipids and membrane proteins (McDonald, 1987). On the other hand, when temperature is too high, membrane become hyper-fluid and destabilization of the lamellar phase occurs, which results in a loss of bilayer integrity (Hazel, 1995; Martínez de Marañón et al., 1999). This alteration in membrane fluidity might impair the activity of some permeases (Abe and Horikoshi, 2000). Therefore, at extreme temperatures, both above or below the physiological range, similar effects can be found in plasma membrane, although by means of different mechanisms. This is because membrane permeases are highly temperature-dependent because changes in temperature can cause conformational changes to their structure (Entian and Barnett, 1992).

Amino acid preferences were also different at both temperatures during the different stages of the fermentation (Table 3). In the first stage of the alcoholic fermentation (25% consumed sugars) the uptake of glycine, methionine, tryptophan and lysine was higher \( (P < 0.05) \) in the fermentation subjected to thermal stress than in the control sample. These amino acids are not preferential nitrogen sources for the yeasts (Boulton et al., 1996). On the other hand, arginine, valine and isoleucine, for which yeasts show more preference, were mainly consumed in the control sample. The uptake of nitrogen by the cells is regulated by the mechanism known as Nitrogen Catabolite Repression (NCR). This NCR enables the cell to select the best nitrogen sources by repressing the transcription of some genes involved in the utilization of poor nitrogen sources (Magasanik, 1992). High temperatures of fermentation would probably affect this regulator mechanism due to the changes produced in plasma membrane, where amino acids transporters are located. The lower consumption of arginine in yeasts subjected to heat stress may be underlined, as this is contrary to what was found in the work of Beltrán and colleagues (2004), where this amino acid was consumed more at low temperatures (13°C). Proline was excreted at the beginning of the fermentation in a similar way in both samples. This amino acid is important in response to stress, because it is accumulated in many bacteria and plant cells as a protectant. Poole and colleagues (2009) observed that the increase in proline accumulation was associated with increased cell viability in conditions of high temperature and osmotic stress. However, it has been shown that proline levels are not increased under various stress conditions in \( S. \) cervevisiae cells (Takagi, 2008). Our results showed higher proline consumption in the control fermentation (ratio C/F35 = 11) than in the samples subjected to thermal stress.

From 25% to 50% consumed sugars, total consumption of amino acids was also higher in control sample than in F35 sample (Table 3). In this stage, arginine uptake was higher in F35 sample than in the control sample. In both samples proline uptake was observed, although more in the control sample. In this sample there was also a greater consumption of asparagine, methionine, leucine, triptophan and lysine. At the end of the fermentation, most of the amino acids were excreted and only proline was consumed, although this consumption was higher in F35 sample than in the control sample.

**Influence of \( SO_2 \) in the utilization of amino acids in samples subjected to thermal stress**

Sulphite acts both on glycolysis and respiratory chain phosphorylation causing ATP depletion. Due to this, cells verify an energy deficiency status, which may require the modifications of some metabolic pathways to gain a more efficient utilization of the energy. At the beginning of the fermentation (25% consumed sugars), the sample that presented the higher total of amino acid consumption was F70 (Table 4); while between the other two samples, consumption was higher in F35 sample than in F0 sample. Arginine uptake was significantly higher \( (P < 0.05) \) in F70 sample than in the other ones, which presented similar results. This amino acid fulfills around 30–50% of the nitrogen requirements of yeasts because it is a major amino acid and its degradation provides three nitrogen atoms from each molecule in anaerobic or fermentative conditions, due to the fact that one atom of nitrogen is usually released as proline (Martín et al., 2003). Lysine and tryptophan, with more than one amino group in the molecule, were consumed equally in all the samples irrespective of the \( SO_2 \) quantity added. Alanine consumption did not show any significant differences with regards to the \( SO_2 \) treatment. Contrary, in previous studies (Garde-Cerdán et al., 2007; Cejudo-Bastante et al., 2010), it was observed that, in normal temperature conditions, this amino acid was highly consumed in fermentations with \( SO_2 \) than in those ones without this additive. The quantity of glutamic acid consumed was significantly higher \( (P < 0.05) \) in the sample with 70 mg \( l^{-1} \) of \( SO_2 \) than in the other ones (Table 4). As regards proline, an important excretion was produced in all three samples at the beginning of the fermentation, although it was greater in F70 sample. Although proline protects cells against many stress conditions including freezing, desiccation, oxidation and ethanol (Takagi, 2008), it does not present a heat-stress-protective activity, so instead of being accumulated in the cytoplasm; it is released outwards as a consequence of arginine metabolism. The rest of amino acids were consumed similarly in all three samples.
From 25% to 50% of fermented sugars, a higher total amino acids were consumed in F70 and F0 samples than in F35 sample (Table 4). Differences regarding total consumption of amino acids during this fermentative phase were mainly due to the high uptake of proline in F70 and F0 samples. During the early stages of wine fermentation, when oxygen may be present, high levels of preferred nitrogen sources result in repression of transporters synthesis and inactivation of existing general amino acid permease and proline specific permease (Soetens et al., 2001). Arginine consumption was higher in F0 and in F35 samples than in F70 sample, so it seems that a high concentration of SO2 difficulted this amino acid transport and, despite being a preferential nitrogen source, its consumption decreased. At the end of fermentation, excretion of most amino acids in the three samples may be underlined, although proline continued to be consumed, especially in F35 sample.

### Experimental procedures

#### Samples and vinification

The grape variety used for this study was *Vitis vinifera* var. Mazuelo. The grapes were destemmed and crushed and afterwards they underwent pressing and filtering. The must was rehydrated in a sterile flask in 12.5 ml of distilled water with 0.125 g of sucrose (number of viable cells per gram \( \approx 2 \times 10^9 \)). It was kept in this medium for 30 min at 29°C. After that, the must was divided into eight aliquots (400 ml each one). The aliquots were inoculated with active dry yeasts *S. cerevisiae* VRB mesophilic strain commercialized by Lallemand (Madrid, Spain). The strains were inoculated in the must in a proportion of 0.25 g l\(^{-1}\). To do this, 1.25 g of dry yeast was rehydrated in a sterile flask in 12.5 ml of distilled water with 0.125 g of sucrose (number of viable cells per gram \( \approx 2 \times 10^9 \)). It was kept in this medium for 30 min at 15°C. The must was inoculated with mixing, in order to get a homogeneous distribution. Two fermentations were carried out at 29°C and 35 mg l\(^{-1}\) of SO2 (the control samples). The other fermentations were subjected to heat stress at 35°C; two fermentations were performed with 0 mg l\(^{-1}\) of SO2 (F0 samples), two fermentation were performed with 35 mg l\(^{-1}\) of SO2 (F35 samples) and two with 70 mg l\(^{-1}\) of SO2 (F70 samples). Fermentations took place in a CO2 trap to allow its exit and tubing system. The first heat exchanger was kept in this medium for 30 min at 29°C and 35 mg l\(^{-1}\) of SO2 (F0 samples), two fermentation were performed with 35 mg l\(^{-1}\) of SO2 (F35 samples) and two with 70 mg l\(^{-1}\) of SO2 (F70 samples). Fermentations took place in 0.5 l round-bottom flasks with a burnished lid with two outlets, one for sample extraction and the other with a CO2 trap to allow its exit and prevent the entrance of air during fermentation. The orifice for sample extraction was covered with a septum during the fermentation. The fermentors were placed over magnetic stirrers (Framo-Geräte technik M21/1, Eisenbach, Germany) at 700 r.p.m., to ensure a homogenous fermentation. The fermentations were carried out in a hot–cold incubator (Selecta, Barcelona, Spain) at a controlled temperature. The fermentations were measured daily for sugar concentration through refractometer ABBE (Misco, Cleveland, USA). Samples were taken before the beginning.
of the fermentation, at 25% of fermented sugars, at 50% of fermented sugars and at the end of fermentation. The concentration of amino acids in the must is presented in Table 5.

Preparation of sample and HPLC analysis of free amino acids

Analyses were performed with a Waters high-pressure liquid chromatograph (Milford, MA, USA) equipped with two 510 pumps, a 717 Plus Autosampler, and a 996 Photodiode Array Detector used at 254 nm. Pico-Tag reverse phase column (300 mm × 3.9 mm i.d.), with a stationary phase of dimethyl-loctadecylsilyl bonded to amorphous silica, was used. The Pico-Tag method used for amino acid analysis is described by Ayestarán and colleagues (1995). Samples were obtained by ultrafiltration with a Millipore Ultrafree MC cartridge (Billerica, MA, USA), and then L-norleucine and L-methionine sulfone (Aldrich, Gillingham, England) were added as internal standards. Afterwars, a precolumn derivatization was carried out with phenylisothiocyanate (Pierce Biotechnology, Rockford, IL, USA).

Empower 2.0 software was employed for chromatographic control. The amount of sample injected was 10 μl. The column was set at 46°C. Mobile phase A: solution of 2.5% (v/v) of acetonitrile (Scharlau, Barcelona, Spain) and 97.5% (v/v) of a solution of sodium acetate (70 mM), with pH adjusted to 6.55 with acetic acid (10%) (Merck, Darmstadt, Germany); mobile phase B: acetonitrile, water and methanol (Scharlau) (48:40:15, v/v/v). The mobile phases used were filtered through a 0.45 μm Millipore filter. Amino acids were eluted under the following conditions: 1 ml min⁻¹ flow rate, elution with linear gradients from 0% to 3% B in 13.5 min, from 3% to 6% B in 10.5 min, from 6% to 9% B in 6 min, from 9% to 34% B in 20 min, maintained during 12 min, from 34% to 100% B in 0.5 min, maintained during 4 min, followed by washing and reconditioning of the column.

Amino acids determinations were performed in quadruplicate on representative samples of the musts. The coefficient of variation for amino acid data obtained by the method described was between 1% and 15%.

Nitrogen fractions and general enological parameters

Amino nitrogen in must (122.5 mg l⁻¹) and general parameters of wine were measured using WineScan 79000 Auto (Foss Analytical, Denmark) with Fourier Transform Infrared Spectroscopy technology. The wavelength range of 240–1295 nm was used for these analyses. Nitrogen fractions and oenological parameters were made in duplicate on the must and wine samples.

Statistical analysis

The statistical study was carried out with analysis of variance (ANOVA) and the means were compared with the Scheffé test. The probability level was 0.05. For data analysis, the software SPSS v.17.0 was used (Chicago, Illinois, USA).

Conclusions

Thermal stress (35°C) produced lower fermentation rates than control fermentation (29°C), and gave rise to wines with higher sugar residue. Amino nitrogen and amino acids consumption was lower in fermentation subjected to thermal stress than in control fermentation. It may be concluded that high fermentation temperatures generated metabolic effects similar to the ones obtained in nitrogen-limited fermentations. Thermal stress also caused differences in amino acids preferences, especially at the beginning of the fermentation. Regarding the effect of SO₂ in fermentations subjected to heat stress, it was found that high doses of SO₂ (70 mg l⁻¹) enhanced the total consumption of amino acids throughout the fermentative process, although these samples did not present the highest fermentative rate. Therefore, it can be stated that high doses of SO₂ protected transport systems of nitrogen better than those of sugars.

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