Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 103 (2007) 771–777
Food Chemistry
www.elsevier.com/locate/foodchem

Influence of SO_2 on the consumption of nitrogen compounds through alcoholic fermentation of must sterilized by pulsed electric fields

Teresa Garde-Cerdán [a], A. Robert Marsellés-Fontanet [b,1], Margaluz Arias-Gil [a,2], Olga Martín-Belloso [b,*,1], Carmen Ancín-Azpilicueta [a,*,2]

[a] Department of Applied Chemistry, Universidad Pública de Navarra, 31006 Pamplona, Spain
[b] CeRTA-UTPV, Food Technology Department, University of Lleida, Avda, Alcalde Rovira Roure, 191, 25198 Lleida, Spain

Received 26 May 2006; received in revised form 3 August 2006; accepted 4 September 2006

Abstract

The aim of this work was to study the influence of SO_2 on the use of nitrogenous compounds by yeast during wine alcoholic fermentation. Thus *Parellada* must was sterilized by a pulsed electric field treatment and inoculated with *Saccharomyces cerevisiae* Na33 strain. The fermentations were carried out with SO_2 (20 mg/l) and without SO_2. Results showed that yeast better consumed the amino acids in the first half of fermentation in the presence of SO_2. The final concentration of amino acids in the obtained wine was greater when the must was fermented without SO_2 than when the latter compound was present. Therefore, the presence of SO_2 facilitated the consumption of amino acids and, hence, such wine should have more complex flavour and better microbiological stability than that obtained from fermentation without SO_2.

Keywords: Sulphur dioxide; Amino acids; Pulsed electric fields (PEF); *Saccharomyces cerevisiae*; Must; Alcoholic fermentation; Wine

1. Introduction

The nitrogen fraction of must is complex and variable and is involved in the kinetics of fermentation and aroma production (Bell & Henschke, 2005; Torrea, Fraile, Garde, & Ancín, 2003) because nitrogen compounds are essential for the growth and metabolism of yeast. However, unless they are completely consumed after the fermentation process, they can promote microbiological instability because of the bacteria growing, and the production of ethyl carbamate, which is a carcinogenic compound (Ough, Crowel, & Mooney, 1988). Some factors affecting the yeast assimilation of nitrogen compounds from the must, such as must composition (Jiranek, Langridge, & Henschke, 1990), must clarification (Ayestarán, Ancín, García, González, & Garrido, 1995), yeast strain (Ough, Huang, An, & Stevens, 1991), pH, temperature and ethanol content (Henschke & Jiranek, 1993) have already been studied. Nevertheless, the effect of a widespread preservative, e.g. sulphur dioxide, on the nitrogen metabolism of yeasts has not yet been reported.

Sulphur dioxide is used in vinification as an antiseptic against undesirable microorganisms and as an antioxidant, against the effects of oxygen. It has also some effect on the activity of certain grape enzymes that promote the loss of quality of the juice and its derivatives, e.g. polyphenoloxidases, including tyrosinase and peroxidase. These enzymes occur in the must, arising from the grape itself, or from fungi that have infected the grapes. SO_2 inactivates these enzymes by reducing their copper cofactor (Romano & Suzzi, 1993). Once dissolved in water, sulphur dioxide is a weak acid that renders other inorganic compounds, generically called SO_2-free forms. In addition, each form

* Corresponding authors. Tel.: +34 948168909; fax: +34 948169606.
E-mail addresses: teresa.garde@unavarra.es (T. Garde-Cerdán), omartin@tecal.udl.es (O. Martín-Belloso), ancin@unavarra.es (C. Ancín-Azpilicueta).
[1] Tel.: +34 973702593; fax: +34 973702596.
[2] Tel.: +34 948168909; fax: +34 948169606.

0308-8146/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2006.09.018

reacts with several components of the must to produce SO_2-combined forms. The concentrations of each product depend mainly on the temperature, pH and the initial concentration of sulphur dioxide since these factors have a strong influence on the equilibrium of the chemical reactions. In general, at low concentrations and high pH, sulphur dioxide has a fungistatic effect whereas, at high concentrations and low pH it acts as a fungicide. It is well accepted that yeasts are mainly affected by SO_2-free forms that disturb essential biological paths after entering the yeast cells. Macris and Markakis (1974) proposed that *Saccharomyces cerevisiae* takes up SO_2 by active transport, although other study (Stratford & Rose, 1986) suggested that diffusion was bound to occur. Once inside the cell, SO_2 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 (Maier, Hinze, & Leuschel, 1986). Thus, the SO_2 could influence in the utilization of amino acids by the yeasts. All SO_2-compounds have antibacterial activity and so it is possible for them to act selectively on the different microorganisms present in must (Hidalgo-Togores, 2002). However, sulphur dioxide could have negative effects on human health (Romano & Suzzi, 1993). For that reason, several international organizations (WHO, FAO, OIV) have set down maximum limits for vinification (350 mg/l) which have caused a reduction of its concentration in foodstuffs, specifically in wines.

Pulsed electric field (PEF) technology has been used to preserve fruit juice and to delay spoilage by microorganisms (Elez-Martínez, Escolà-Hernández, Soliva-Fortuny, & Martín-Belloso, 2004, 2005; Sen Gupta, Masterson, & Magee, 2005). Recently, this technology has been implemented for the production of commercial fruit juices in the USA whereas some important food processors of the EU are trying this technology at a pilot plant level. A previous study showed that a PEF treatment did not affect the contents of nitrogen compounds and fatty acids of must (Garde-Cerdán, Arias-Gil, Marsellés-Fontanet, Ancín-Azpilicueta, & Martín-Belloso, in press). This is an important issue since these compounds are essential for the development of the yeasts during fermentation. It has also been reported that PEF treatments decrease the activities of enzymes such as peroxidases and polyphenoloxidases in apple and pear extracts (Giner, Gimeno, Barbosa-Cánovas, & Martín, 2001), peach puree (Giner et al., 2002) and orange juice (Elez-Martínez, Aguiló-Aguayo, & Martín-Belloso, 2006). The proposed mechanism of enzymatic inactivation might be related to the change of specific structures of the enzymes (Zhong et al., 2007). However, as far as we know, the combination of both PEF and SO_2 has not been considered previously in grape juice processing.

Due to the fact that a PEF treatment allows reduction of the level of sulphur dioxide to guarantee the biochemical and microbiological stability of the must, the aim of this study was to assess the effect of the sulphur dioxide content on the consumption of nitrogen compounds throughout the alcoholic fermentation of must processed by PEF. Thus this study could be a starting point for an effective reduction of the sulphur dioxide content of wines.

2. Materials and methods

2.1. Must sterilization

A local wine manufacturer, Raimat (Lleida, Spain), kindly provided grapes (*Vitis vinifera* L. variety *Parellada*). They were harvested at ripeness and then, washed, drained, split from bunches and frozen at −20 °C prior to processing. The grapes were thawed at 5 °C for 24 h; afterwards they were manually pressed, yielding 12 l of must that was de-aerated by stirring under reduced pressure for 30 min.

A laboratory scale PEF unit (Ohio State University, Columbus, OH, USA) was used to treat must. The pulse generator module consisted of a high voltage generator (OSU-4F), which could supply differences of electric potential between the electrodes of the treatment chambers of up to 12 kV, and a pulse generator unit model 9412A (Quantum Composers, Inc., Bozeman, MT, USA), which could render square wave pulses of up to 10 μs and 2000 Hz. PEF treatment was performed with bipolar electric field pulses of 4 μs width and with an electric field strength of 35 kV/cm. The pulse repetition rate was 1000 Hz and the total PEF treatment time was 1 ms, which was calculated as the product of the pulse width and the number of pulses delivered to the must (Garde-Cerdán et al., in press).

2.2. Vinification

The PEF-processed must was divided into four batch of 3 l. Diammonium phosphate (DAP) was added to the must until it reached approximately 55 mg N/l, due to the low ammonium content of the must. Two lots of must were kept without any preservative and the other two lots were supplied with potassium metabisulphite up to a concentration of 20 mg/l of total SO_2. After that, all musts were inoculated with an active dry form of the Na33 strain of *S. cerevisiae* subsp. *cerevisiae* selected from the Estación de Viticultura y Enología de Navarra (Olite, Spain) and commercialized by Lallemand España. Na33 strain was inoculated into the musts in a proportion of 0.2 g/l, rehydrating 0.65 g of dry yeast in a sterile flask with 7.5 ml of distilled water containing 0.07 g of sucrose for 30 min at 35 °C (number of viable cells/ml $\geqslant 2 \times 10^9$).

The fermentations took place in glass fermentors with a capacity of 3.5 l and with a burnished lid with two outlets, one of them for sample extractions and the other with a CO_2 trap to eliminate it from the fermentative environment and prevent the entrance of air during fermentation. The hole for sample extraction was covered with a septum during the fermentation. The fermentors were placed over magnetic stirrers (Ikamag RCT basic, Milian SA, Geneve, Switzerland) at 630 rpm, to ensure

a homogeneous fermentation. The fermentations were carried out in a hot-cold incubator (Selecta, Barcelona, Spain) at a controlled temperature of 18 °C. The fermentations were daily measured for sugar concentration through their refractive index at 20 °C, using a refractometer ABBE model 325 (Misco, Cleveland, OH, USA) and through enzymatic measures (reagents from Chema Italia, Rome, Italy) using a multi-parametric analyzer Enochem (Tecnología Difusión Ibérica, Barcelona, Spain). This is an automated device where the appropriate reactions take place. It automatically provides the necessary reactants and also performs the spectrophotometric measurement of the absorbance changes after the programmed incubation time. Samples were taken before the beginning of the fermentation, at 50% of consumed sugars and at the end of fermentation (reducing sugars <2.5 g/l). All recipients and materials, which were in contact with the samples, were previously sterilized.

2.3. Analysis of free amino acids

Analyses were performed with a Waters high-pressure liquid chromatography (Milford, MA, USA) equipped with two 510 pumps, a 717 Plus Autosampler, and a 996 Photodiode Array Detector used at 254 nm. A Pico·Tag reverse phase column (300 mm × 3.9 mm i.d.), with a stationary phase of dimethyloctadecylsilyl bonded to amorphous silica, was used. Amino acid derivatization was performed using a Waters Pico·Tag workstation. The Pico·Tag method used for amino acid analysis is described by Ayestarán et al. (1995). Samples were cleaned 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. Afterwards, a precolumn derivatization was carried out with phenylisothiocyanate (Pierce Biotechnology, Rockford, IL, USA). A Millennium 32 software package (Waters) 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% of acetonitrile (Scharlau, Barcelona, Spain) and 97.5% 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) (45:40:15, 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 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 the column. Amino acid determination was repeated four times for each sample. The concentrations of amino acids are given as the mean values and the standard deviations of eight analyses since the fermentations were carried out in duplicate.

2.4. Nitrogenous fractions and oenological parameters

The ammonium content was analysed by enzymatic measurement of the ammonium cation present in the samples with the multi-parametric analyzer Enochem, using reactives from Chema Italia. The free amino acid content measured by HPLC was taken as the amino nitrogen value, whereas the assimilable nitrogen was calculated as the sum of the ammonium and the amino nitrogen, without taking into account the proline concentration. All the measurements were performed four times.

Determinations of acetic acid, total SO_2, acetaldehyde and total polyphenols were done in the multi-parametric analyzer Enochem by enzymatic and colorimetric methods. The pH was determined using a pH-meter Metrohm 702 (Metrohm, Herisau, Germany). The total acidity was determined, following the method described by the Office International de la Vigne et du Vin (1990). The alcoholic level of the final wine was determined by using a Salleron-Dujardin ebulliometer (Paris, France). All the fermentations and analyses were done in duplicate so that the values shown in Table 1 are the mean values of four analyses.

Table 1
Enological parameters at the beginning (PEF treated must), at 50% of consumed sugars and of the final wines obtained from fermentation with SO_2 (20 mg/l) and without SO_2

Sample	pH	Acetic acid (g/l)	Total acidity (g/l[a])	Total SO_2 (mg/l)	Acetaldehyde (mg/l)	Total polyphenols (mg/l)	Alcohol (v/v %)
Must							
	3.83 ± 0.02	–	2.43 ± 0.05	19.5 ± 0.2	4 ± 0	250 ± 3	–
50% of consumed sugars							
With SO_2	3.22 ± 0.04	–	3.5 ± 0.2	13 ± 1	17.5 ± 0.2	203 ± 4	4.2 ± 0.2
Without SO_2	3.17 ± 0.02	–	4.1 ± 0.1	–	11 ± 1	190 ± 0.6	4.1 ± 0.1
Wine							
With SO_2	3.24 ± 0.02	–	4.1 ± 0.1	8 ± 1	1.7 ± 0.5	122 ± 2	8.3 ± 0.2
Without SO_2	3.25 ± 0.01	–	4.33 ± 0.07	–	8 ± 1	122 ± 4	8.5 ± 0.5

All parameters are given with their standard deviation ($n = 4$).
[a] Expressed as g/l tartaric acid.

3. Results and discussion

3.1. Oenological parameters

The wines obtained from the inoculated must fermented, with or without SO_2, did not show the presence of acetic acid (Table 1). This result could be due to the fact that the inoculated yeast (*S. cerevisiae* Na33 strain) produces a very low concentration of acetic acid (Fraile, 2002). In general, *Saccharomyces* yeasts produce less acetic acid than do other yeasts (Rojas, Gil, Piñaga, & Manzanares, 2003).

The concentration of acetaldehyde was higher at the beginning of the fermentation in the samples fermented in the presence of SO_2 than in those fermented without SO_2, which is known to enhance the production of acetaldehyde (Herraiz, Martin-Alvarez, Reglero, Herraiz, & Cabezudo, 1989). At the end of the fermentation, a higher concentration of acetaldehyde was observed in the wine obtained from the fermentation without SO_2 (Table 1).

During the alcoholic fermentation, the concentration of total polyphenols decreased in the studied samples. Polyphenols (including catechins, proanthocyanidins, cinnamic acids and their derivatives) are subject to oxidation so that the initial straw yellow colour of white wines turns into the deep golden yellow typical of browned wines (Margheri, Tonon, & Trepin, 1980). The wines obtained from both fermentations, with or without SO_2, presented similar levels of total polyphenols (Table 1); therefore, the SO_2 content did not affect the final polyphenol concentration.

3.2. Nitrogenous fractions

The ammonium nitrogen of the initial must represented 17% of the assimilable nitrogen (Table 2). This fact could promote an increase of higher alcohols because the yeasts are forced to use the amino acids of must as nitrogen source (Usseglio-Tomasset, 1998). The highest uptake of all nitrogen fractions occurred in the first half of the fermentation, likely due to the exponential growth phase of yeast where nitrogen is used for biomass production (O'Connor-Cox & Ingledew, 1989). The ammonium nitrogen was almost entirely consumed, either with SO_2 (96%) or without SO_2 (94%). Amino nitrogen was reduced by up to a 76% when the fermentation was carried out with sulphur dioxide and 67% in the absence of SO_2 (Table 2). Thus, the total consumption of assimilable nitrogen was 328 mg N/l (95%) during the fermentation carried out with SO_2 and 315 mg N/l (91%) when SO_2 was not present (Table 2).

3.3. Must composition and utilization of amino acids during the fermentation

Arginine and proline, together with alanine, were the most abundant amino acids in must (Table 3). They accounted for 68% of the total amino acids content of must. This fact agrees with data reported by other authors (Ancín-Azpilicueta, Fraile-Jiménez de Maquirriain, Garde-Cerdán, & Torrea-Goñi, 2005; Henschke & Jiranek, 1993). The concentration of arginine in the initial must is by far the greatest, accounting for 56% of total amino acids. This result is similar to those reported by other authors (Valero, Millán, Ortega, & Mauricio, 2003). *Parellada* variety had a low proline/arginine ratio, which means that the must contained a high quantity of assimilable nitrogen for the yeasts. This ratio is mainly influenced by the grape variety, and the grape ripeness (Bell & Henschke, 2005).

It is well known that the availability and metabolism of nitrogen compounds are important for fermentation development and final wine quality. As can be seen in Table 3, amino acids are taken up by *S. cerevisiae* mainly during the first half of the fermentation. Moreover, in this phase, the consumption of amino acids by yeasts was greater in the fermentation carried out with SO_2 (1018 mg/l) than in that performed without this substance (867 mg/l). The most consumed amino acid was the most abundant, that is arginine; this amino acid presented a higher consumption in the fermentation carried out in the presence of SO_2 (689 mg/l) than in the fermentation without SO_2 (596 mg/l) (Table 3). Arginine and ammonium ions were the yeasts' principal source of nitrogen during fermentation. Alanine was consumed to a great extent by yeasts during both fermentations (with SO_2, 54.2 mg/l; without SO_2, 50.8 mg/l). Leucine, threonine, tryptophan, tyrosine, isoleucine, γ-amino butyric

Table 2
Nitrogenous fractions of the initial PEF treated must, at 50% of consumed sugars and of the final wines obtained from fermentation with SO_2 (20 mg/l) and without SO_2

Samples	Ammonium nitrogen (mg N/l)	Amino nitrogen (mg N/l)	Assimilable nitrogen (mg N/l)
Must			
	57.9 ± 0.5	300 ± 1	347 ± 1
50% of the consumed sugars			
With SO_2	3.7 ± 0.7	129 ± 1	27 ± 1
Without SO_2	5 ± 0	175 ± 4	68 ± 8
Wine			
With SO_2	2.3 ± 0.6	71 ± 3	18.8 ± 0.6
Without SO_2	3.7 ± 0.7	100 ± 6	31.7 ± 0.7

All parameters are given with their standard deviation ($n = 8$).

Table 3
Concentration of amino acids (mg/l) in the initial PEF treated must, at 50% of consumed sugars and in the final wines obtained from fermentation with SO_2 (20 mg/l) and without SO_2

Amino acids	Must	50% of consumed sugars		Wine	
		With SO_2	Without SO_2	With SO_2	Without SO_2
Proteic amino acids					
Arginine (Arg)	692 ± 21	3.0 ± 0.1	96 ± 7	3.03 ± 0.09	13 ± 1
Proline (Pro)	89 ± 3	874 ± 33	919 ± 40	446 ± 12	591 ± 25
Alanine (Ala)	58 ± 2	3.8 ± 0.1	7.2 ± 0.2	8.7 ± 0.2	11.9 ± 0.7
Leucine (Leu)	30 ± 1	–	–	–	1.2 ± 0.1
Serine (Ser)	28 ± 2	3.1 ± 0.1	2.7 ± 0.2	2.4 ± 0.4	2.5 ± 0.6
Threonine (Thr)	26 ± 4	–	–	–	–
Tryptophan (Trp)	23.5 ± 0.2	–	–	–	–
Glutamic acid (Glu)	22.5 ± 0.8	21 ± 1	34 ± 2	22.1 ± 0.2	22 ± 1
Aspartic acid (Asp)	21.7 ± 0.8	3.2 ± 0.1	8.2 ± 0.5	4.2 ± 0.2	5.3 ± 0.3
Valine (Val)	21 ± 2	–	1.7 ± 0.1	–	1.5 ± 0.2
Asparagine (Asn)	20.4 ± 0.8	2.8 ± 0.1	7.4 ± 0.3	3.7 ± 0.3	7.2 ± 0.2
Tyrosine (Tyr)	16.3 ± 0.6	–	–	–	2.0 ± 0.1
Histidine (His)	16 ± 1	1.7 ± 0.3	–	1.9 ± 0.1	2.6 ± 0.2
Methionine (Met)	15 ± 1	–	5.3 ± 0.3	–	4.0 ± 0.2
Phenylalanine (Phe)	14.8 ± 0.3	0.81 ± 0.01	–	–	0.45 ± 0.03
Isoleucine (Ile)	10.3 ± 0.3	–	–	–	–
Lysine (Lys)	5 ± 1	8 ± 1	4.8 ± 0.4	3.0 ± 0.1	3.1 ± 0.2
Glycine (Gly)	3.7 ± 0.3	4.8 ± 0.2	16 ± 2	3.0 ± 0.2	7.0 ± 0.7
Non-proteic amino acids					
γ-Amino butyric acid (Gaba)	35 ± 3	–	–	–	–
Cystathionine (Cyst)	30 ± 1	15 ± 1	21 ± 2	11 ± 1	13 ± 1
Creatinine (Creat)	19 ± 2	27 ± 1	36 ± 1	18.7 ± 0.1	27 ± 1
Citrulline (Cit)	11.1 ± 0.4	–	–	–	–
Ornithine (Orn)	10.2 ± 0.7	1.9 ± 0.1	11 ± 1	1.0 ± 0.1	8 ± 1
Hydroxyproline (Hyp)	6.9 ± 0.7	22 ± 2	18 ± 1	0.76 ± 0.03	2.6 ± 0.1
Phosphoserine (Pser)	6.1 ± 0.2	6.7 ± 0.1	5.9 ± 0.1	2.9 ± 0.1	2.4 ± 0.2

The concentrations are shown with their standard deviations ($n = 8$).

acid, and citrulline were completely consumed in this phase of fermentation in both cases since they are suitable nitrogen sources (Henschke & Jiranek, 1993). Glutamic acid was not consumed in either of the two fermentations though this amino acid is a preferred source for the yeasts (Table 3). This phenomenon could be attributed to the fact that the arginine consumption was very high and glutamate anion is among the final products of arginine metabolism, so yeasts did not need to take glutamic acid from the medium. The lysine and glycine contents did not decrease in this step of the fermentations because these amino acids are not considered good nitrogen sources for *S. cerevisiae*, although they could be metabolized by microorganisms in other fermentations (Cooper, 1982). Yeasts did not consume proline during the initial stage of fermentation because this amino acid is taken up by the yeasts only under severe nitrogen stress conditions and the presence of ammonium in the medium will inhibit or repress the uptake of proline (Ough & Stashak, 1974). The liberation of proline to the medium might be due to the metabolism of arginine since it is an intermediate product in the degradation of arginine (Ough et al., 1988). It is thought that, at the beginning of fermentation, *S. cerevisiae* obtains the necessary nitrogen compounds for cellular division from the medium without performing any modification on them. Once the first necessities of each amino acid are satiated, yeasts usually take those amino acids in excess in the medium to use them as nitrogen source (Bisson, 1991).

In the second half of fermentation, the amino acid uptake was the same in both types of fermentation (466 mg/l), so that the concentration of SO_2 did not affect to the amino acid consumption in this phase of fermentation. The total decrease of amino acids was lower than that achieved in the first part of the fermentation (Table 3). Conversely to the first stage, proline was the most consumed amino acid, accounting for 49% (428 mg/l) and 36% (328 mg/l) of the total in the fermentation with and without SO_2, respectively (Table 3). The yeasts could use this amino acid in this phase of the fermentation since there were less good nitrogen sources in the medium than at the beginning of the fermentation (Table 3). When the good nitrogen sources were depleted, the general amino acid permeases and the specific permeases, such as proline permease, allow the accumulation of poorer nitrogen sources such as proline (Bell & Henschke, 2005). The remaining amino acids underwent few variations in their concentrations, except arginine, glutamic acid and glycine, which showed significantly reduced concentrations in the fermentation without SO_2, and alanine, which was excreted in both types of fermentation (Table 3). Non-proteic amino acids were consumed to greater extents through the second half of the fermentation (Table 3).

At the end of the fermentation, high ethanol concentration usually alters the structure and permeability of the plasmatic cell membrane, accelerating the passive entry of protons as the electrochemical gradient between both membrane faces decreases in a similar way (Cartwright et al., 1986). While this process takes place, some yeasts also release amino acids into the wine by a passive process of desorption. All these processes are the physiological response to the exhaustion of sugars (Bidan, Feuillat, & Moulin, 1986). However, the yeast strain used in our study was resistant to the presence of ethanol, which is a specific strain characteristic (Jones, 1989). Thus, the yeasts did not release amino acids at the end of the fermentation but they continued to consume amino acids. At the end of the vinification process, the wine obtained from the fermentation with SO_2 had a lower concentration of amino acids (532 mg/l) than had the wine obtained from the fermentation without SO_2 (728 mg/l).

4. Conclusions

The consumption of amino acids was higher when the fermentation of the must was performed with sulphur dioxide. SO_2 content affected to the consumption of total amino acids in the first phase of fermentation. Arginine was the most abundant amino acid in the must and it was also the most consumed amino acid in the fermentation. Its consumption was favoured in the fermentation carried out in the presence of sulphur dioxide. Throughout the second part of fermentation, the most consumed amino acid was proline and the presence of SO_2 also enhanced its consumption. The final content of amino acids in the wine obtained from the fermentation without SO_2 was greater than that measured in the wine obtained from the fermentation with this compound. Therefore, it appears that the presence of sulphur dioxide promoted the consumption of total amino acids and hence the wine obtained from the fermentation with SO_2 should have more complex flavour and better microbiological stability than that obtained from the fermentation without SO_2.

Acknowledgements

This work was supported by the *Ministerio de Ciencia y Tecnología* through the project AGL2002-04399-C02-02. A.R. Marsellés-Fontanet would like to thank the *Ministerio de Ciencia y Tecnología* for his pre-doctoral grant.

References

Ancín-Azpilicueta, C., Fraile-Jiménez de Maquirriain, P., Garde-Cerdán, T., & Torrea-Goñi, D. (2005). Influence of inoculation of selected yeast on the quality of rosé and white wines. In A. P. Riley (Ed.), *Food research, safety and policies* (pp. 57–92). New York: Nova Science Publishers, Inc.

Ayestarán, B., Ancín, C., García, A., González, A., & Garrido, J. (1995). Influence of prefermentation clarification on nitrogenous contents of musts and wines. *Journal of Agricultural and Food Chemistry, 43*, 476–482.

Bell, S.-J., & Henschke, P. A. (2005). Implications of nitrogen nutrition for grapes, fermentation and wine. *Australian Journal of Grape and Wine Research, 11*, 242–295.

Bidan, P., Feuillat, M., & Moulin, J. P. (1986). Les vins mousseux. Rapport francais. 65ème Assemblée Générale de l'OIV. *Bulletin de l'OIV, 663–664*, 563–626.

Bisson, L. F. (1991). Influence of nitrogen on yeast and fermentation of grapes. In J. Rantz (Ed.), *Proceedings of the international symposium on nitrogen in grapes and wine* (pp. 78–89). Davis: American Society for Enology and Viticulture.

Cartwright, C. P., Juroszek, J. R., Beaven, M. J., Ruby, F. M. S., de Morais, S. M. F., & Rose, A. H. (1986). Ethanol dissipates the protonmotive force across the plasma membrane of *Saccharomyces cerevisiae*. *Journal of General Microbiology, 132*, 369–377.

Cooper, T. G. (1982). Transport in *Saccharomyces cerevisiae*. In J. N. Strathern, E. W. Jones, & J. B. Broach (Eds.), *The molecular biology of the yeast saccharomyces. Metabolism and gene expression* (pp. 399–461). New York: Cold Spring Harbor Laboratory.

Elez-Martínez, P., Aguiló-Aguayo, I., & Martín-Belloso, O. (2006). Inactivation of orange juice peroxidase by high-intensity pulsed electric fields as influenced by process parameters. *Journal of the Science of Food and Agriculture, 86*, 71–81.

Elez-Martínez, P., Escolà-Hernández, J., Soliva-Fortuny, R. C., & Martín-Belloso, O. (2004). Inactivation of *Saccharomyces cerevisiae* suspended in orange juice using high-intensity pulsed electric fields. *Journal of Food Protection, 67*, 2596–2602.

Elez-Martínez, P., Escolà-Hernández, J., Soliva-Fortuny, R. C., & Martín-Belloso, O. (2005). Inactivation of *Lactobacillus brevis* in orange juice by high-intensity pulsed electric fields. *Food Microbiology, 22*, 311–319.

Fraile, P. (2002). Influencia de cepas seleccionadas de *S. cerevisiae* en la composición volátil de vinos rosados y blancos. Evolución durante la fermentación. Thesis. Universidad Pública de Navarra, Spain.

Garde-Cerdán, T., Arias-Gil, M., Marsellés-Fontanet, A. R., Ancín-Azpilicueta, C., & Martín-Belloso, O. (in press). Effects of thermal and non-thermal processing treatments on fatty acids and free amino acids of grape juice. *Food Control, 18*.

Giner, J., Gimeno, V., Barbosa-Cánovas, G. V., & Martín, O. (2001). Effects of pulsed electric fields processing on apple and pear polyphenoloxidases. *Food Science and Technology International, 7*, 339–345.

Giner, J., Ortega, M., Mesegué, M., Gimeno, V., Barbosa-Cánovas, G. V., & Martín, O. (2002). Inactivation of peach polyphenoloxidase by exposure to pulsed electric fields. *Journal of Food Science, 67*, 1467–1472.

Henschke, P. A., & Jiranek, V. (1993). Metabolism of nitrogen compounds. In G. H. Fleet (Ed.), *Wine microbiology and biotechnology* (pp. 77–164). Victoria: Harwood Academic Publishers.

Herraiz, T., Martin-Alvarez, P. J., Reglero, G., Herraiz, M., & Cabezudo, M. D. (1989). Differences between wines fermented with and without sulphur dioxide using various selected yeasts. *Journal of the Science of Food and Agriculture, 49*, 249–258.

Hidalgo-Togores, J. (2002). *Tratado de Enología*. Madrid: Mundi-Prensa.

Jiranek, V., Langridge, P., & Henschke, P. A. (1990). Nitrogen requirement of yeast during wine fermentation. In P. J. Williams, D. M. Davidson, & T. H. Lee (Eds.), *Proceedings of the seventh Australian wine industry technical conference* (pp. 166–171). Adelaida: Australian Industrial Publishers.

Jones, R. P. (1989). Biological principles for the effects of ethanol. *Enzyme and Microbial Technology, 11*, 130–153.

Macris, B. J., & Markakis, P. (1974). Transport and toxicity of sulphur dioxide in *Saccharomyces cerevisiae* var. *ellipsoideus*. *Journal of the Science of Food and Agriculture, 25*, 21–29.

Maier, K., Hinze, H., & Leuschel, L. (1986). Mechanism of sulfite action on the energy metabolism of *Saccharomyces cerevisiae*. *Biochimica et Biophysica Acta, 848*, 120–130.

Margheri, G., Tonon, D., & Trepin, P. (1980). I polifenoli dei vini bianchi come potenziali di ossidazione. *Vignevini, 7*, 35–44.

O'Connor-Cox, E. S. C., & Ingledew, W. M. (1989). Wort nitrogenous sources. Their use by brewing yeasts: a review. *The Journal of the American Society of Brewing Chemists, 47*, 102–108.

Office International de la Vigne et du Vin. (1990). Recueil des Méthodes Internationales d'Analyse des Vins et des Moûts. Paris, France.

Ough, C. S., Crowel, E. A., & Mooney, L. A. (1988). Formation of ethyl carbamate precursors during grape juice (chardonnay) fermentation. I. Addition of amino acid, urea, and ammonia: effects of fortification on intercellular and extracellular precursors. *American Journal of Enology and Viticulture, 39*, 243–249.

Ough, C. S., Huang, Z., An, D., & Stevens, D. (1991). Amino acid uptake by four commercial yeasts at two different temperatures of growth and fermentation: effects on urea excretion and reabsorption. *American Journal of Enology and Viticulture, 42*, 26–40.

Ough, C. S., & Stashak, R. M. (1974). Further studies on proline concentration in grapes and wines. *American Journal of Enology and Viticulture, 25*, 7–12.

Rojas, V., Gil, J. V., Piñaga, F., & Manzanares, P. (2003). Acetate ester formation in wine by mixed cultures in laboratory fermentations. *International Journal of Food Microbiology, 86*, 181–188.

Romano, P., & Suzzi, G. (1993). Sulfur dioxide and wine microorganisms. In G. H. Fleet (Ed.), *Wine microbiology and biotechnology* (pp. 373–393). Victoria: Harwood Academic Publishers.

Sen Gupta, B., Masterson, F., & Magee, T. R. A. (2005). Inactivation of *E. coli* in cranberry juice by a high voltage pulsed electric field. *Engineering in Life Sciences, 5*, 148–151.

Stratford, M., & Rose, A. H. (1986). Transport of sulphur dioxide by *Saccharomyces cerevisiae*. *Journal of General Microbiology, 132*, 1–6.

Torrea, D., Fraile, P., Garde, T., & Ancín, C. (2003). Production of volatile compounds in the fermentation of chardonnay musts inoculated with two strains of *Saccharomyces cerevisiae* with different nitrogen demands. *Food Control, 14*, 565–571.

Usseglio-Tomasset, L. (1998). *Química Enológica*. Madrid: Mundi-Prensa.

Valero, E., Millán, C., Ortega, J. M., & Mauricio, J. (2003). Concentration of amino acids in wine after the end of fermentation by *Saccharomyces cerevisiae* strains. *Journal of the Science of Food and Agriculture, 83*, 830–835.

Zhong, K., Wu, J., Wang, Z., Chen, F., Liao, X., Hu, X., & Zhang, Z. (2007). Inactivation kinetics and secondary structural change of PEF-treated POD and PPO. *Food Chemistry, 100*, 115–123.