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Influence of addition of ammonium and different amino acid concentrations on nitrogen metabolism in spontaneous must fermentation

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Abstract

The effect of the addition of different amino acid concentrations in must on yeast nitrogen metabolism during alcoholic fermentation was studied. To do this, fermentations of *Mazuelo* must, poor in nitrogen compounds, were carried out. Ammonium and different concentrations of amino acids (0, 45, 120, 250 and 450 mg/l) were added to the must. Addition of 45, 120 and 250 mg/l of proteic amino acids to the must increased the rate of fermentation. Proline was mainly consumed in fermentations with smaller amounts of amino nitrogen and, at the same time, this amino acid showed the highest residual concentration in the final wines. The consumption of other proteic amino acids was directly proportional to their concentration in the musts, with the exception of leucine and isoleucine that were synthesized. However, a difference in the percentages of the amino acids consumed by the yeasts was observed. The percentages of aspartic acid, alanine and arginine consumed were higher in the fermentations supplemented with amino acids than in the fermentation where only ammonium was added. The percentages of tyrosine and phenylalanine consumed gradually increased with increase of their initial concentration.

Keywords: Amino acids; Nitrogen metabolism; Addition of nitrogen compounds; *Mazuelo* must; Wine

1. Introduction

Nitrogen composition of the must has an important effect on rate of fermentation because nitrogen compounds increase biomass production and stimulate the rate of sugar utilization, avoiding stuck and sluggish fermentations. Furthermore, amino acids are precursors of volatile compounds and, consequently, wine composition is affected by the content of the amino acids in the must. Both, soil poor in nitrogen compounds and early grape harvests favour low amino acid contents in must. To avoid this, it is usual to fertilize the soil, although this does not always assure sufficient content of nitrogen compounds in the grape (Bell & Henschke, 2005). Moreover, this cultural practice could create some problems as the high vineyard nitrogen application results in reduced grape colour and total soluble solids concentration (Kliewer, 1977). Another way to avoid these problems is to add nutritional supplements to the must before fermentation, usually inorganic forms of nitrogen, such as ammonium salts. Furthermore, the addition of mixtures of ammonium and amino acids could improve fermentation, as the amino acids can be incorporated directly into the yeast protein synthesis.

Few studies exist on the effect of addition of amino acids to the must on nitrogen metabolism of yeasts during alcoholic fermentation. Most studies have investigated the effect of ammonium as a nitrogen source (Agenbach, 1977; Marks, van der Merwe, & van Vuuren, 2003). In addition, the studies of addition of amino acids and their effect on the metabolism of yeast starting culture have

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mainly used synthetic media (Beltran, Esteve-Zarzoso, Rozés, Mas, & Guillamón, 2005). In the present work, the effect of adding ammonium salts and amino acids, at various concentrations to a must poor in nitrogen compounds (*Mazuelo* variety) was studied. The aim was to examine the metabolism of nitrogen in spontaneous fermentations of the must and to evaluate the role and contribution of the nitrogen sources added. To do so, spontaneous fermentations of must of *Mazuelo* variety were carried out. The same quantity of ammonium and different concentrations of amino acids were added to the must.

2. Materials and methods

2.1. Samples and vinification

The grape variety used was *Vitis vinifera* var. *Mazuelo*. The grape was destemmed and crushed and afterwards it underwent pressing and filtering. Potassium metabisulphite (Aldrich, Gillingham, UK) was added to the must up to a concentration of 35 mg/l of total SO_2. To carry out the study, the must was divided into five lots. Diammonium phosphate (Aldrich) was added to one lot, as the only nitrogen source, up to a concentration of 113 mg N/l of ammonium nitrogen (F1). This same amount of ammonium was added to four other samples and different quantities of total proteic amino acids, 45 mg/l (F2), 120 mg/l (F3), 250 mg/l (F4) and 450 mg/l (F5). Each proteic amino acid was added in the same proportion as they were found in the initial must (F1) (Table 1). To do so, each proteic amino acid (Aldrich) was weighed on an analytical balance with an absolute weight error of 0.0005 mg. Afterwards, the amino acids were added to the must, well-mixed to ensure complete dissolution. The concentrations of each amino acid in the initial must (F1) and in the musts supplemented with proteic amino acids (F2–F5) are shown in Table 1.

The fermentations were carried out in glass fermentors with a capacity of 600 ml and with a burnished lid with two outlets, one for sample extractions and the other with a CO_2 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 (Ikamag RCT basic, Milian SA, Geneve, Switzerland) that work at 630 rpm, to ensure a homogeneous fermentation. The fermentors were placed in a hot–cold incubator set at 24 °C (Selecta, Barcelona, Spain). The fermentations were measured daily for sugar concentration by refractive index at 20 °C, using a refractometer, ABBE model 325 (Misco, Cleveland, OH, USA). The sugar concentration to determine the end of fermentation was measured using an enzymatic kit (reactives from Chema Italia, Rome, Italy) using a multi-parametric analyser Enochem (Tecnología Difusión Ibérica, Barcelona, Spain). All fermentations were conducted in duplicate. The duration of each of the fermentations is shown in Table 3. Samples of the must and of the wines were taken at the end of all fermentations (reducing sugars <2.5 g/l). All recipients and materials that were in contact with the samples were previously sterilized using a mixture of peracetic acid and hydrogen peroxide (Henkel, Barcelona, Spain).

Table 1
Concentration of proteic amino acids in initial *Mazuelo* must and in the different samples supplemented with NH_4^+ and proteic amino acids

Proteic amino acids	Concentration of amino acids (mg/l)				
	Initial must with NH_4^+ (F1)	Must with NH_4^+ and 45 mg/l of amino acids (F2)	Must with NH_4^+ and 120 mg/l of amino acids (F3)	Must with NH_4^+ and 250 mg/l of amino acids (F4)	Must with NH_4^+ and 450 mg/l of amino acids (F5)
Proline (Pro)	184 ± 7	212	254	340	462
Aspartic acid (Asp)	6.9 ± 0.3	7.9	9.3	12.1	16.1
Arginine (Arg)	8.7 ± 0.4	12.2	17.6	28.4	43.8
Alanine (Ala)	20.6 ± 0.9	24.6	30.8	43.2	61.9
Valine (Val)	3.4 ± 0.2	3.9	4.7	6.3	8.6
Methionine (Met)	3.8 ± 0.3	4.2	4.9	6.2	8.2
Tyrosine (Tyr)	1.04 ± 0.05	1.1	1.3	1.6	2.0
Phenylalanine (Phe)	2.8 ± 0.1	3.2	3.6	4.5	5.9
Threonine (Thr)	1.7 ± 0.1	2.0	2.3	3.1	4.2
Asparagine (Asn)	7.0 ± 0.3	4.4	5.8	8.6	12.6
Glycine (Gly)	2.0 ± 0.1	2.3	3.0	4.3	6.2
Tryptophan (Trp)	4.0 ± 0.4	4.6	5.7	7.8	10.7
Cysteine (Cys)	1.4 ± 0.1	1.6	1.9	2.5	3.4
Lysine (Lys)	5.1 ± 0.6	6.3	8.1	11.8	17.2
Glutamic acid (Glu)	25 ± 1	27.7	32.2	41.2	54.2
Serine (Ser)	6.3 ± 0.4	7.4	9.0	12.7	16.9
Isoleucine (Ile)	0.51 ± 0.05	0.6	0.7	0.9	1.2
Leucine (Leu)	0.44 ± 0.05	0.5	0.6	0.8	1.0
Total amino acids	285 ± 7	326	396	536	736

2.2. Preparation and HPLC analysis of free amino acids

Analysis was performed with a Waters high-pressure liquid chromatograph (Waters Chromatography Division, Milford, MA) equipped with two 510 pumps, a 717 Plus Autosampler, and a 996 Photodiode Array detector used at 254 nm. The Pico·Tag method used is described by Ancín, Ayestarán, and Garrido (1996). All the analyses were done four times. The coefficients of variation for amino acid data, obtained by the method described, were between 5% and 15%.

2.3. Nitrogenous fractions and enological parameters

Ammonium nitrogen was quantified using an enzymatic kit (Chema Italia), using the multi-parametric analyser Enochem. These analyses were made four times. Amino nitrogen was calculated by determining free amino acids by HPLC and assimilable nitrogen was calculated as the sum of ammonium nitrogen and amino nitrogen minus the proline nitrogen.

Determinations of volatile acidity, total SO_2 and acetaldehyde were done using the multi-parametric analyser Enochem by enzymatic and colorimetric methods. The pH was determined using a pH-meter, Metrohm 702 (Metrohm, Herisau, Germany). The total acidity was determined by the method described by the Office International de la Vigne et du Vin (1990). The alcoholic level of the final wine was determined with a Salleron–Dujardin ebullimeter (Paris, France). These determinations were done in duplicate.

3. Results and discussion

3.1. Characteristics of initial must, final wines and kinetics of fermentation

Table 2 shows that the pH values of all the wines were below 3.6, a value considered limiting for correct product conservation. Volatile acidity was practically the same in all the wines under study, except in the wine obtained from fermentation where 450 mg/l of amino acids were added (F5). This wine showed a volatile acidity of 0.7 mg/l. This could be because this fermentation was slower than the other fermentations (Table 3). The addition of amino acids have no influence on the alcoholic level reached in the wines (Table 2). The wine obtained from the fermentation where 450 mg/l of amino acids were added to the must (F5), showed a concentration of acetaldehyde somewhat higher than that in the wines obtained from the other fermentations (F1–F4). This could be because the lower rate of this fermentation meant that the must was less protected from oxygen.

To characterize the kinetics, fermentation rate was calculated from fermentation curves as an average percentage of the daily-consumed sugar in the range 0–99% of total sugars (vf 0–99) (Houtman & du Plessis, 1985). These results are shown in Table 3. The addition of 45, 120 and 250 mg/l of amino acids to the must improved the fermentative kinetics to an important extent, as the duration of these fermentations was from 6–7 days less than the fer-

Table 3
Features of the fermentation kinetics in the samples

	dt_{0-99}[a] (days)	vf_{0-99}[b] (%/day)
Fermentation 1	20	4.95
Fermentation 2	14	7.07
Fermentation 3	14	7.07
Fermentation 4	13	7.62
Fermentation 5	27	3.67

F1: supplemented with ammonium. F2: supplemented with ammonium and 45 mg/l of proteic amino acids. F3: supplemented with ammonium and 120 mg/l of proteic amino acids. F4: supplemented with ammonium and 250 mg/l of proteic amino acids. F5: supplemented with ammonium and 450 mg/l of proteic amino acids.

[a] Days needed to ferment from 0% to 99% of sugars.

[b] Average percentage of sugar used daily during the fermentation time required from 0% to 99% of the total.

Table 2
Characteristics of initial must and final wines

	pH	Volatile acidity (g/l)[a]	Total acidity (g/l)[b]	Total SO_2 (mg/l)	Alcohol (v/v%)	Acetaldehyde (mg/l)	Ammonium nitrogen (mg N/l)	Amino nitrogen (mg N/l)	YAN (mg N/l)
Initial must	2.99 ± 0.02	–	10 ± 1	35 ± 0	–	–	17.0 ± 0.5	45 ± 2	40 ± 2
Wines									
F1	2.92 ± 0.04	0.4 ± 0.1	8.1 ± 0.5	15 ± 6	11.38 ± 0.04	13.3 ± 3	–	28 ± 1	15 ± 1
F2	2.92 ± 0.04	0.46 ± 0.04	8.5 ± 0.2	21 ± 1	11.2 ± 0.1	17.2 ± 2	–	35 ± 3	15 ± 3
F3	2.92 ± 0.06	0.46 ± 0.04	8.3 ± 0.4	18 ± 5	11.2 ± 0.1	18 ± 1	–	42 ± 2	18 ± 2
F4	2.92 ± 0.03	0.4 ± 0.0	8.4 ± 0.3	19 ± 0	11.2 ± 0.1	13.5 ± 0.7	–	50 ± 1	15 ± 1
F5	2.91 ± 0.03	0.7 ± 0.2	9.0 ± 0.4	13 ± 1	11.2 ± 0.1	20.5 ± 0.7	–	72 ± 2	21 ± 2

The results of enological parameters and nitrogen fractions are shown with their standard deviation ($n = 4$ and $n = 8$, respectively).

F1, wine from must supplemented with ammonium; F2, wine from must supplemented with ammonium and 45 mg/l of proteic amino acids; F3, wine from must supplemented with ammonium and 120 mg/l of proteic amino acids; F4, wine from must supplemented with ammonium and 250 mg/l of proteic amino acids; and F5, wine from must supplemented with ammonium and 450 mg/l of proteic amino acids. YAN, yeast assimilable nitrogen.

[a] As g/l acetic acid.

[b] As g/l tartaric acid.

mentation in which only ammonium was added (Table 3). However, in this range of amino acid concentrations, the rates of fermentation were similar, independently of the concentration of amino acids added. Hernández-Orte, Ibarz, Cacho, and Ferreira (2005, 2006) did not find significant differences in the rates of fermentation after adding different quantities of amino acids to the must. When 450 mg/l of proteic amino acids were added to the must, the fermentation rate was below the rates of the other fermentations.

3.2. Must composition and nitrogen fractions

Table 1 shows the concentrations of proteic amino acids in the five samples of *Mazuelo* musts (F1–F5). The total concentration of the proteic amino acids in the initial must was 285 mg/l, a value much lower than that found in other musts from different viticultural regions (Henschke & Jiranek, 1993). Proline, alanine, and glutamic acid were the amino acids that were present at the highest concentration in the must, making up 81% of the total proteic amino acids. Several authors have found that these amino acids, along with arginine, are the most abundant in must from different varieties of grape grown in different viticultural regions (Henschke & Jiranek, 1993). Among the majority amino acids, the high concentration of proline stands out, and it was the most abundant amino acid in the initial must. The ratio between proline and arginine is influenced mainly by the grape variety and level of grape maturity (Bell & Henschke, 2005). For the *Mazuelo* must, this ratio was above 1. The rest of the amino acids were found at low concentrations, below or on the lower limit of the ranges found by other authors for different musts (Conradie, 2001; Miele, Carbonneau, & Bouard, 2000; Hernández-Orte, Guitart, & Cacho, 1999).

Yeast assimilable nitrogen (YAN) was calculated as the sum of ammonium nitrogen and amino nitrogen minus proline nitrogen. The initial YAN of the must was 40 mg N/l (Table 2). This concentration was below the necessary concentration of assimilable nitrogen (140 mg N/l) to ensure a suitable alcoholic fermentation (Agenbach, 1977). The concentration of ammonium nitrogen in the ini-

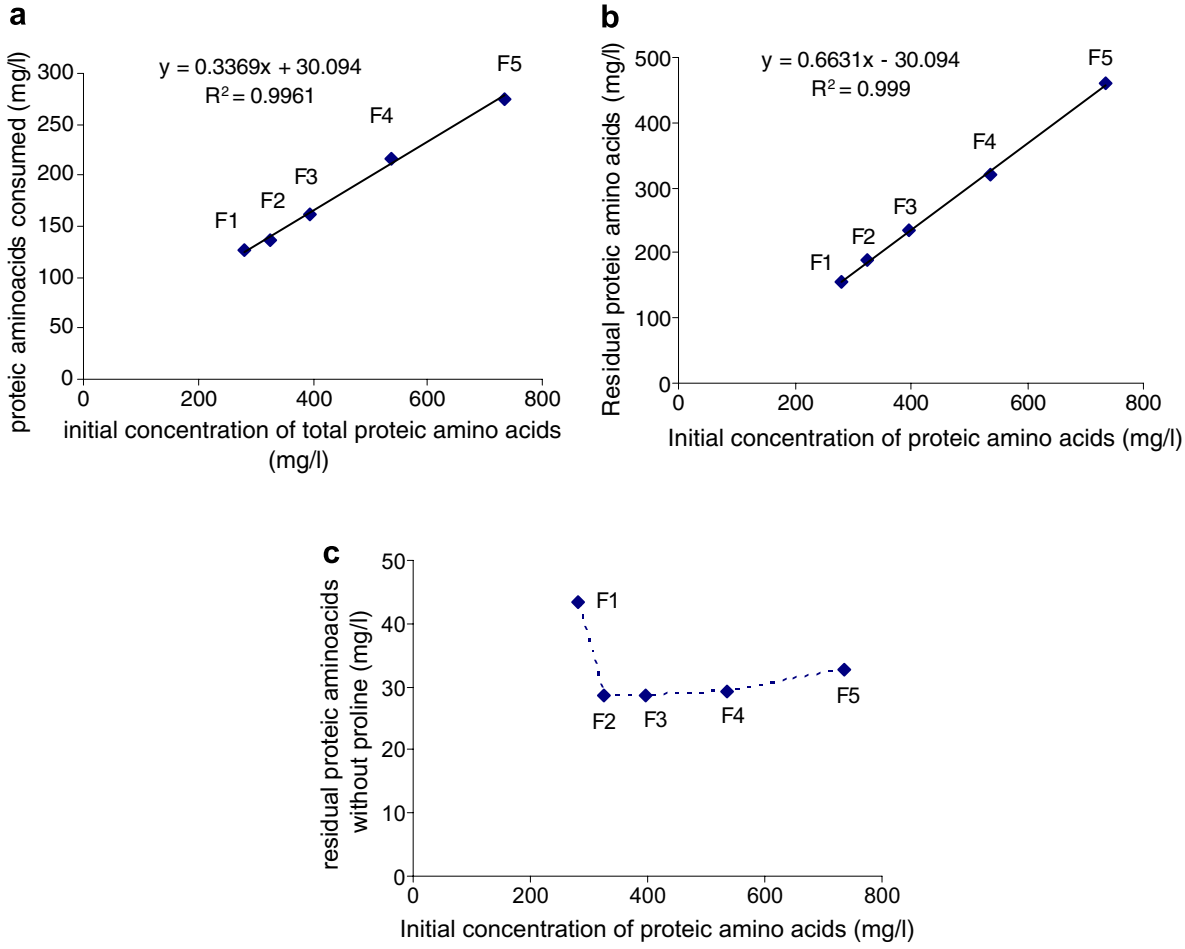

Fig. 1. (a) Concentration (mg/l) of proteic amino acids consumed during fermentation; (b) concentration (mg/l) of residual proteic amino acids in wines; (c) concentration (mg/l) of residual proteic amino acids in wines, less the concentration of proline. Wine from must supplemented with: F1, ammonium; F2, ammonium and 45 mg/l of proteic amino acids; F3, ammonium and 120 mg/l of proteic amino acids; F4, ammonium and 250 mg/l of proteic amino acids; and F5, ammonium and 450 mg/l of proteic amino acids.

tial must represented 24.4% of nitrogen, while the amino nitrogen represented 72.6% of the total nitrogen. In all fermentations, the ammonium nitrogen was totally consumed, even in the fermentations supplemented with amino acids (Table 2). Ammonium nitrogen serves as the primary available nitrogen form for normal yeast metabolism and, as such, its concentration is rapidly reduced when fermentation begins (Zoecklein, Fugelsang, Gump, & Nury, 1990). The concentration of residual amino nitrogen in the wines increased as the initial concentration of the amino acids in the different musts increased (Table 2). This did not occur with yeast assimilable nitrogen, as the YAN values in the wines were similar, independently of the initial concentration of amino acids in the musts.

3.3. Consumption of amino acids in fermentations

During fermentation, the yeasts consumed a higher quantity of proteic amino acids as the concentration of these increased in the medium (Fig. 1a). The consumption of proteic amino acids showed a positive correlation ($R^2 = 0.9961$) with the concentration of initial amino acids in the must. On the other hand, the consumption of amino acids was not complete in any of the fermentations under study (Fig. 1b). The concentrations of residual amino acids in the wines were directly proportional to the concentrations of amino acids in the initial must ($R^2 = 0.999$) (Fig. 1b).

Without taking into account the proline, in the fermentations where 45, 120, 250 and 450 mg/l of proteic amino acids were added, the concentrations of residual amino acids were similar (Fig. 1c). In the fermentation where only ammonium was added, the concentration of residual amino acids was above the residual amino acids of the other fermentations. Comparing Fig. 1a and b it can be concluded that proline was the most abundant residual amino acid in the fermentations supplemented with amino acids. The amino acids constitute a source of nitrogen and carbon for residual microorganisms that have been able to survive at the end of the fermentation. Consequently, high concentrations of amino acids in wines can have a negative effect on microbiological stability.

Table 4 shows the quantity (mg/l) as well as the percentage of proteic amino acids consumed in the different fermentations (F1–F5). The quantity of proline consumed was greater in the fermentation without the addition of amino acids (F1), so that the consumption of proline was favoured in the poorest nitrogen medium. This coincides with results found by Brandriss and Magasanik (1979) and Ough and Stashak (1974). When good nitrogen sources are scarce in the medium, increases in general amino permease (GAP) and PUT (proline permease) activity have been observed (Bell & Henschke, 2005). It would seem that, with fermentations poor in nitrogen sources (F1), proline permease activity was important. The quantity of proline consumed was practically the same in the fermentations where 45, 120 and 250 mg/l of amino acids were added to the must (F2, F3 and F4). In the fermentation where 450 mg/l of amino acids were added (F5) the quantity of proline consumed was less than those in the rest of the fermentations. As regards the percentage of proline consumed, this decreased although the initial concentration

Table 4
Consumption (mg/l, %) of proteic amino acids in fermentations of *Mazuelo* musts supplemented with ammonium and different concentrations of amino acids ($n = 8$)

	mg/l of amino acid consumed					% of amino acid consumed				
	F1	F2	F3	F4	F5	F1	F2	F3	F4	F5
Proline	72.7	50.7	49.1	50.2	34.7	39.6	24.0	19.3	14.8	7.5
Aspartic acid	5.5	7.4	8.8	11.6	15.2	79.3	93.4	94.3	95.4	94.2
Arginine	3.9	10.3	15.7	26.4	38.7	44.9	84.6	89.2	93.0	88.3
Alanine	16.4	20.6	26.8	38.3	56.5	79.9	83.9	87.2	88.6	92.7
Valine	2.7	3.9	4.7	6.3	8.6	79.4	100	100	100	100
Methionine	2.7	3.7	4.4	5.7	7.7	72.5	89.4	89.6	91.4	94.3
Tyrosine	−1.4[a]	0.02	0.3	0.4	0.9	−135.2	1.8	19.9	26.1	45.2
Phenylalanine	−0.3	0.9	1.4	2.3	3.6	−8.8	28.1	37.6	51.0	60.5
Threonine	1.0	1.4	1.8	3.1	4.2	57.0	70.9	75.9	100	100
Asparagine	0.2	1.4	3.0	4.7	9.2	6.6	32.3	52.2	55.3	72.7
Glycine	0.9	1.3	1.9	3.7	4.6	48.4	56.7	65.0	85.1	73.8
Tryptophan	4.0	4.5	5.7	7.8	10.7	100	96.7	100	100	100
Cysteine	1.4	1.6	1.9	2.5	3.4	100	100	100	100	100
Lysine	5.1	6.3	8.1	11.8	17.2	100	100	100	100	100
Glutamic acid	17.3	17.5	21.5	31.6	44.7	70.1	63.2	66.7	76.7	82.4
Serine	5.5	6.3	8.3	10.8	16.1	86.6	85.4	91.9	88.1	95.3
Isoleucine	−9.1	−0.9	−0.9	−0.6	−0.7	–	–	–	–	–
Leucine	−2.6	−0.5	−0.5	−0.01	−0.03	–	–	–	–	–

F1, fermentation supplemented with ammonium; F2, fermentation supplemented with ammonium and 45 mg/l of proteic amino acids; F3, fermentation supplemented with ammonium and 120 mg/l of proteic amino acids; F4, fermentation supplemented with ammonium and 250 mg/l of proteic amino acids; and F5, fermentation supplemented with ammonium and 450 mg/l of proteic amino acids.

[a] The negative data are excretions.

of proline in the must increased (Table 4). When the proline concentration was increased in the *Mazuelo* musts, the concentrations of the other proteic amino acids also increased, so that the yeasts consumed other amino acids considered as good nitrogen sources, instead of proline.

The consumptions of the other proteic amino acids were proportional to their initial concentrations in the musts (Table 4), except for isoleucine and leucine, which were synthesized. However, differences in the consumed percentage of the amino acids by the yeasts were observed.

In the fermentation to which only ammonium was added (F1), the consumed percentages of aspartic acid, arginine, alanine, valine and methionine were below those of the rest of the fermentations, which had been supplemented with amino acids (F2–F5). However, when different concentrations of amino acids were added (F2–F5), the percentage of these amino acids consumed was independent of the initial concentration added to the must (Table 4). The percentage of these amino acids consumed was approximately 80% when only ammonium was added to the must and it came close to 90–100% when amino acids were added to the must. Arginine was the exception, as its percentage consumption in the fermentation to which only ammonium was added, was about 45%. These results suggest that a limit of concentration existed, beyond which the consumption of aspartic acid, arginine, alanine, valine and methionine by the yeast was favoured. It is likely that, in poor nitrogen mediums, the specific permeases present in the yeasts, for the assimilation of these amino acids, showed less activity than in the must supplemented with amino acids and consequently richer in nitrogen sources. The low consumed percentage of arginine in the fermentation where only ammonium was added could be due to the high consumption of proline as a nitrogen source in this fermentation. Proline is an intermediate in the catabolism of arginine for the formation of glutamic acid or ketoglutaric acid and ammonium (Brandriss & Magasanik, 1980). As the yeasts consumed and probably degraded proline directly to glutamic acid, they did not need to consume large quantities of arginine to obtain glutamic acid.

Tyrosine and phenylalanine were excreted in the fermentation where only ammonium had been added to the must (F1) and in the rest of the fermentations (F2–F5) these amino acids were consumed (Table 4). In F1, these amino acids were probably synthesized by the yeasts for their proteic synthesis as they were found in very low concentrations in the must. The excess of tyrosine and phenylalanine formed by the yeasts was probably excreted into the medium. Other authors have observed excretions of amino acids in must poor in nitrogen compounds and in a medium with ammonium as the only nitrogen source (Delisle & Phaff, 1961). Furthermore, the concentration of tyrosine excreted into the medium was higher than that of phenylalanine. This is probably because the formation of tyrosine in the interior of the cell is favoured compared to the formation of phenylalanine (Jones & Fink, 1982). In the fermentations supplemented with proteic amino acids, the percentages of tyrosine and phenylalanine consumed increased in a gradual way with an increase in the initial concentrations of these amino acids in the musts (Table 4). Beltran, Novo, Rozés, Mas, and Guillamón (2004) found that increase in the concentration of aromatic and branched-chain amino acids, favours the formation and activation of their specific permeases (Tat1p and Bap2p, respectively). Consequently, the gradual increases of the percentages of tyrosine and phenylalanine consumed, as their concentration increases in the medium, could be because both the formation and activity of their specific permeases were favoured. The consumption of threonine, asparagine and glycine increased in general, in a gradual manner in the fermentations as the concentration of these amino acids increased in the musts (Table 4). From what has been observed in this study, the specific permeases for the consumption of threonine, asparagine and glycine could have followed the same pattern. Afterwards, the degree of utilization of these amino acids was a function of their concentrations in the medium.

Tryptophan, cysteine and lysine were totally consumed in the five fermentations, independently of the amount of amino acids added to the medium (Table 4). Lysine is not considered a good nitrogen source for *Saccharomyces* yeasts (Henschke & Jiranek, 1993). However, as the fermentations under study were spontaneous, it is likely that the non-*Saccharomyces* yeasts, present at the initial stage of fermentation, consumed this amino acid, as lysine is a good nitrogen source for these yeasts (Large, 1986). Unlike what happened with the other two aromatic amino acids, tyrosine and phenylalanine, tryptophan was totally consumed in all fermentations, independently of the concentration of amino acids added to the must (Table 4). Consequently, it may be concluded that the specific permease of the yeasts for the assimilation of aromatic amino acids (Tat1p) could present a greater affinity for tryptophan than for tyrosine or phenylalanine.

The profile of consumed percentage of glutamic acid and serine follows an irregular tendency, and in both cases it was lower in the fermentation to which 45 mg/l of amino acids were added (F2) than in the other fermentations (F1, F3–F5) (Table 4). These amino acids showed high consumed percentages, between 63.2% and 82.4% for glutamic acid and between 85.4% and 95.3% for serine. This may be because both amino acids are good nitrogen sources for the yeasts (Henschke & Jiranek, 1993).

Isoleucine and leucine were synthesized and excreted in all the fermentations under study (Table 4), although to a greater extent in the fermentation where only ammonium was added as nitrogen source (F1). Although the yeasts probably consumed them at the start of fermentation, as they were in low concentrations in the musts, the quantity consumed may not have been sufficient for proteic synthesis. Consequently, the yeasts would have had to synthesize them throughout the fermentation and, at the end of vinification, the excess of isoleucine and leucine formed was excreted into the medium.

4. Conclusions

The addition of 45, 120 and 250 mg/l of amino acids to the must improved the fermentative kinetics to an important extent. However within this range of concentrations, the fermentation rate was independent of the concentrations of amino acids added to the must. Furthermore, it was observed that the greater the concentrations of amino acids added, the greater were their consumptions during fermentation. The most abundant residual amino acid in the wines was proline. The consumption of proteic amino acids was directly proportional to their initial concentration in the musts, with the exceptions of leucine and isoleucine, which were synthesized. However, differences were observed in the percentages of the amino acids consumed by the yeasts. The percentages of aspartic acid, alanine and arginine, all good nitrogen sources consumed, were independent of the concentrations of amino acids added to the must and they were consumed in lower percentages when the fermentations were not supplemented with amino acids. The percentage of aromatic amino acids consumed gradually increased with increase in the initial concentration of these in the medium, except for tryptophan, which was totally consumed in all the fermentations.

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