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Journal of Food Engineering 66 (2005) 315–322
JOURNAL OF FOOD ENGINEERING
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Free amino acids and biogenic amines in wines and musts from the Alentejo region. Evolution of amines during alcoholic fermentation and relationship with variety, sub-region and vintage

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Received 14 October 2003; accepted 18 March 2004

Abstract

Characterization of musts and wines from different Alentejo sub-regions, varieties and vintages was made in terms of amino acid and amine contents, by HPLC with fluorescence detection of OPA/FMOC derivatives. The evolution of volatile and biogenic amines was also studied throughout 10 microvinifications.

No significant increase in the levels of total volatile amines was observed during alcoholic or spontaneous malolactic fermentation. While higher histamine levels were only found during the storage period, an increase in the concentration of tyramine was confirmed in red wines immediately after malolactic fermentation, which seems to be also the main origin of putrescine.

It was noticed that grape variety, region of production, and vintage can influence free amino acid and amine contents of musts and wines, although alcoholic and malolactic fermentations can override that effect.

Keywords: Amino acids; Amines; Alcoholic fermentation; Variety; Region; Vintage

1. Introduction

The presence of amines in musts and wines is well documented in the literature (Ough, Daudt, & Crowell, 1981; Mafra, Herbert, Santos, Barros, & Alves, 1999; Radler & Fäth, 1991; Zee, Simard, L'Heureux, & Tremblay, 1983), being biogenic amines of particular importance (Bauza et al., 1995). However, the processes that generate these amines, together with the factors that influence their quantitative and qualitative presence are in some cases not well defined yet and, sometimes, agreement is lacking between the published results.

Amines in wine may have two different sources: raw materials and fermentation processes. Some amines are already found in grapes, namely histamine and tyramine (Vidal-Carou, Ambatlle-Espunyes, Ulla-Ulla, & Mariné-Font, 1990), as well as several volatile amines and polyamines (Feuillat, 1998; Radler & Fäth, 1991).

Other studies showed that both alcoholic (Buteau, Duitschaever, & Ashton, 1984) and malolactic fermentations (Aerny, 1985; Kállay & Bódy-Szalkai, 1996; Vidal-Carou, Codony-Salcedo, & Mariné-Font, 1990) may produce amines in wines. Rivas-Gonzalo, Santos-Hernandez, and Mariné-Font (1983) and Vidal-Carou et al. (1990), stated that at least part (and sometimes all) of the final content of tyramine in the tested wines was due to alcoholic fermentation, although not confirming an increase in the histamine content during this process, nor finding any increase in histamine or tyramine levels during malolactic fermentation in 50% of the studied wines. However, Quevauviller and Mazière (1969) and Buteau et al. (1984) reported production of histamine during alcoholic fermentation, although Vidal-Carou et al. (1990) observed a highly significant correlation

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doi:10.1016/j.jfoodeng.2004.03.024

between levels of histamine and tyramine and lactic acid–malic acid ratio for red wines and a significant correlation in white and rosé wines.

Work concerning the factors that influence type and quantity of amines in wines, particularly biogenic, indicates lactic bacteria as the main cause for the significant generation of these substances, particularly some strains of *Lactobacillus* and *Pediococcus* (Moreno-Arribas, Torlois, Joyeux, Bertrand, & Lonvaud-Funel, 2000; Radler & Fäth, 1991); *Saccharomyces* species were shown to be weak producers of histamine by Lafon-Lafourcade and Joyeux (1975). However, some *Oenococcus oeni* strains possess decarboxylase activity capable of producing amines (Leitão, Teixeira, Crespo, & San Romão, 2000; Lonvaud-Funel & Joyeux, 1994), yeast extracts may contain noticeable amounts of histamine and tyramine (Blackwell, Mabbitt, & Marley, 1969), and some "non-*Saccharomyces*" yeasts (these may proliferate in the early stages of alcoholic fermentation) are capable of producing histamine at concentrations as high as 8.3 mg/l (Abad & Gómez, 1987). The presence of *Botritis cinerea* in grapes appears to influence both quantity and quality of amines in musts (Hajos, Sass-Kiss, Szerdahelyi, & Bardocz, 2000). Amongst others, vintage and technology employed (Sass-Kiss, Szerdahelyi, & Hajos, 2000), variety of grape (Soleas, Carey, & Goldberg, 1999), levels of amine-precursor amino acids in musts (Bauza, Blaise, Daumas, & Cabanis, 1995; Soufleros, Barrios, & Bertrand, 1998), and assimilable nitrogen content (Soufleros et al., 1998) seem to be relevant factors.

In this work, the levels of amines and assimilable amino acids in wines were studied regarding grape variety, region and vintage. To complement the work, an attempt was made to study the changes not only in the biogenic amine contents (histamine, tyramine, tryptamine, β-phenylethylamine, including the polyamines putrescine and cadaverine) but also in volatile amines during the alcoholic and malolactic fermentations.

2. Material and methods

2.1. Samples

A set of 209 samples from the Demarcated Region of Alentejo (including different sub-regions) was studied, comprising monovarietal wines from 1997 (20 samples) and 1998 (18 samples), monovarietal musts from 1998 (19 samples) and 1999 (20 samples), as well as the samples collected during the fermentations, that led to 1998 wines from the Évora sub-region (47 red and 85 white musts fermentations, collected at different stages of fermentation). To study the effect of variety, region and vintage, the following sampling scheme was employed: Monovarietal wines were produced by microvinification, with the main cultivars from the Alentejo region: Arinto, Perrum, Antão Vaz, Rabo de Ovelha and Roupeiro for white, and Aragonez, Moreto, Castelão, Tinta Caiada and Trincadeira for red. Trincadeira and Roupeiro came from the following Alentejo sub-regions: Évora, Portalegre, Borba, Reguengos, Redondo and Vidigueira, all the other cultivars being from Évora.

To study the evolution of biogenic amine contents during fermentation, only the samples from 1998 were used.

The volume obtained in each microvinification was about 40 l without temperature control, for red wine, and 20 l for white wine, fermented at 17 °C. Red grapes fermentation was conducted in steel microvinificators, without yeast inoculation and stems, in the presence of skin and seeds. White grapes fermentation was carried out in glass containers, with no yeast inoculation, stems, skins or seeds. Average length of white fermentation was 41 days (45 maximum and 34 minimum). At the beginning of alcoholic fermentation, 100 mg/l of SO_2 were added to both red and white musts, and another 50 mg/l after spontaneous malolactic fermentation completion in red wines, and after alcoholic fermentation completion in white wines. All red wines and Rabo de Ovelha white wine from 1998 showed spontaneous malolactic fermentation. The collected samples were immediately frozen and kept at −15 °C until analysis.

After the beginning of alcoholic fermentation, red and white wines were kept in glass containers (20 l) for ca. seven months at room temperature and ±17 °C, respectively. This can be considered a current procedure in the production of Alentejo wines, although white wines are usually bottled earlier (after three or four months). Following this period they were bottled and kept at room temperature until analysis. During the stage in glass containers, red and white wines were checked for free sulfur dioxide levels.

2.2. Analytical methods

Free amino acids (aspartate, glutamate, asparagine, glutamine, alanine, arginine, histidine, glycine, phenylalanine, tyrosine, tryptophan, γ-aminobutyric acid, serine, lysin, threonine, methionine, leucine, isoleucine, valine, proline and two intermediates of the urea cycle, ornithine and citrulline) and amines (ethanolamine, methylamine, ethylamine, histamine, tyramine, β-phenylethylamine, tryptamine, isoamilamine, cadaverine and putrescine) analyses were performed according to a previously developed HPLC method with fluorescence detection of the OPA/FMOC derivatives (Herbert, Santos, & Alves, 2001). Precision of this method ranged from 0.6% to 11.6% (relative standard deviation (%), RSD) for a standard solution (with an average amino acids concentration of 2.75 mg/l and an average amines

concentration of 1.4 mg/l) and from 0.5% to 19.2% for wine samples (mean: 6.0% for volatile amines, 10.6% for biogenic amines, 6.1% for assimilable amino acids—all amino acids except proline, 6.5% for proline). Average accuracy, calculated by the standard addition method, was 99.8% (coefficient of variation 11.1%; $n = 6$), with isoamylamine (81%) and histamine (138%) as minimum and maximum values, respectively.

All the other parameters, namely °Brix, free and total sulfur dioxide, total and volatile acidity, alcohol content, reducing sugars and pH were determined following the OIV (Office International de la Vigne et du Vin, 1990) methods.

2.3. Waste handling

In this work, the produced waste was recovered whenever possible, in order to reuse certain substances, diminish the toxicity of others and properly store all residues. The procedure adopted for residues from chromatographic analysis of free amino acids and amines was described in previous work (Herbert et al., 2001).

2.4. Statistics

Tests of significance for the analytical methodology were accomplished employing analysis of variance (ANOVA) and Student's *t*-test, using Statgraphics® Plus v. Windows 1.4 1995 (Manugistics, Maryland, USA).

3. Results and discussion

The chemical characterization of the samples studied is shown in Tables 1 and 2. The reducing sugar content of both red and white musts was similar, as were total acidity and pH (Table 1). As expected, red wines had lower levels of free and total sulfur dioxide than white (Table 2). In both red and white wines, the alcohol content was similar, although Trincadeiras and Roupeiros from the different sub-regions had slightly higher ethanol content than the varietal average from Évora. The reducing sugar contents were similar among all wines studied. Red wines had higher volatile acidity and pH than white ones, and this may be explained by the occurrence of malolactic fermentation in all red wines.

3.1. Evolution of amines during alcoholic fermentation

Changes in concentration for each amine during alcoholic fermentation of each of the 10 musts from the 1998 vinification were studied. Five red musts were fermented (Aragonez, Moreto, Castelão, Tinta Caiada and Trincadeira) and so were five white musts (Arinto, Perrum, Antão Vaz, Rabo de Ovelha and Roupeiro), all from the same sub-region—Évora. It was noticed that spontaneous malolactic fermentation occurred in all red wines, and in Rabo de Ovelha white wine. The white wine Roupeiro from Portalegre 1998 (whose fermentation was not studied) showed spontaneous malolactic fermentation.

Table 1
Chemical characterization of 1998/1999 musts (mean ± SD)

Parameter	°Brix	Reducing sugars (g/l)	Total acidity (g/l)	pH
Red from Évora ($n = 10$)	21.6 ± 1.8	213 ± 20	4.5 ± 0.9	3.8 ± 0.2
White from Évora ($n = 10$)	21.2 ± 1.8	206 ± 20	4.7 ± 1.2	3.6 ± 0.3
Trincadeira from sub-regions ($n = 10$)	22.0 ± 1.1	216 ± 13	5.3 ± 0.5	3.6 ± 0.1
Roupeiro from sub-regions ($n = 12$)	21.5 ± 0.9	210 ± 10	5.0 ± 0.7	3.7 ± 0.1

SD—standard deviation, *n*—number of samples.
Note: Samples corresponding to Roupeiro and Trincadeira from Évora are included simultaneously in the musts from Évora group and in the musts from sub-regions group.

Table 2
Chemical characterization of 1997/1998 wines (mean ± SD)

Parameter	Sulfur dioxide (mg/l)		Alcohol (% v/v)	Reducing sugars (g/l)	Acidity (g/l)		pH
	Free	Total			Volatile	Titrable	
Red from Évora ($n = 10$)	27.0 ± 9.4	84.6 ± 20.7	12.4 ± 0.8	2.1 ± 0.5	0.55 ± 0.08	3.32 ± 0.60	4.0 ± 0.2
White from Évora ($n = 10$)	34.0 ± 4.8	112 ± 11.8	12.6 ± 0.6	2.7 ± 1.1	0.18 ± 0.12	4.12 ± 0.46	3.6 ± 0.3
Trincadeira from sub-regions ($n = 10$)	23.4 ± 11.6	89.4 ± 19.8	12.8 ± 1.0	2.4 ± 0.6	0.50 ± 0.07	3.74 ± 0.22	3.9 ± 0.1
Roupeiro from sub-regions ($n = 12$)	37.8 ± 6.8	108.0 ± 11.2	13.3 ± 0.6	2.3 ± 1.4	0.31 ± 0.07	3.87 ± 0.36	3.7 ± 0.1

SD—standard deviation, *n*—number of samples.
Note: Samples corresponding to Roupeiro and Trincadeira from Évora are included simultaneously in the musts from Évora group and in the musts from sub-regions group.

For most of the samples, cadaverine, tryptamine, β-phenylethylamine and isoamylamine amines, were found to be below the limit of detection for the method used (0.28 mg/l for amines, on average). During alcoholic and malolactic fermentations the levels of ethanolamine had no significant changes. In general, no remarkable increase in the levels of total volatile amines was seen during alcoholic or malolactic fermentations.

Average methylamine concentrations of wines were lower than those initially found in the musts, which indicates the reduction of methylamine concentration during fermentation. This reduction seems to be more pronounced for the red varieties studied. Possible explanations include the volatility of the compound and/or its metabolization during fermentation, although this fact remains unproven. However, the highest fermentation temperatures of red wines could contribute to higher evaporation rates comparing to white ones.

The concentration of ethylamine in white musts is similar in the beginning and in the end of the fermentation, with no drastic changes during the process. For red varieties, with initial average values (mean ± standard deviation) in musts of 2.7 ± 2.2 mg/l, ethylamine concentrations increased, on average, during alcoholic fermentation (maximum 23 mg/l). However, after 18 months of storage, the average concentration was 4.3 ± 3.2 mg/l. In these cases, the production of ethylamine during fermentation may have counterbalanced the losses by evaporation. Three of the five red grape varieties studied showed higher final concentrations of ethylamine than any of the white wines studied.

Concerning the biogenic amines (histamine, tyramine, tryptamine, β-phenylethylamine, putrescine and cadaverine), it was noted that their concentration did not vary greatly during alcoholic fermentation, particularly in relation to histamine, which showed levels close to the values initially found in the musts (average 1.2 mg/l for red musts).

Generally, there was no significant change in the levels of histamine during either alcoholic or malolactic fermentations, being close to the values initially found in musts. However, samples taken about 18 months after the completion of malolactic fermentation in red wines, displayed consistently higher levels of histamine (11.1 mg/l for red and 12.5 mg/l for white wines), than the corresponding musts. Regarding the histidine decarboxylase in 23 strains of lactic acid bacteria (*O. oeni*, *Lactobacillus brevis* and *Lactobacillus* spp.), Campo, Lavado, Duenas, and Irastorza (2000), stated that the accumulation of histamine in the semi-synthetic medium by the producing strains reached its highest after 10–15 days of growth, that maximum being delayed by 8 days when maximum bacterial growth was achieved. It is possible that a similar effect could have occurred for the vinifications studied here. Nevertheless, the production of histamine after malolactic fermentation during the storage period cannot be neglected.

Our results showed increasing levels of histamine for all sampled wines as can be seen in Fig. 1 for red and Fig. 2 for white, respectively. However, malolactic fermentation occurred only in red wines and Rabo de Ovelha white wine. Moreover, it was noted that red wine had the lowest histamine level. It would have been desirable to sample the bottled wines regularly throughout the 18 months before final sampling, in order to show the rate of histamine production during storage.

The evolution of tyramine concentrations was different in red and white varieties. At the end of fermentation, white wines showed levels of tyramine (0.2 mg/l on average) close to those initially present in musts (0.1 mg/l on average), with no increase in concentration during fermentation for these musts. Identical results were observed for putrescine levels in white wines. The profile was different for red varieties (Fig. 3), which initially presented low tyramine levels in musts, and higher values than any of the white varieties at the end. While higher histamine levels were found only in the storage period, an increase in tyramine concentration was confirmed in all 5 vinifications immediately following malolactic fermentation. These results seem to be in

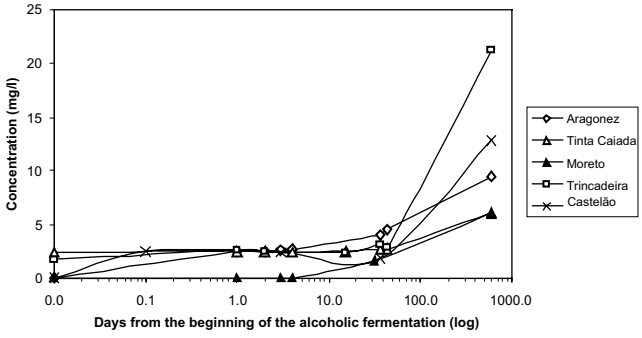

Fig. 1. Evolution of histamine concentration for red grape varieties during fermentation.

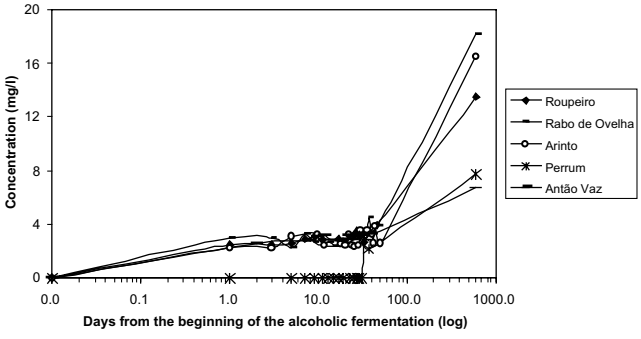

Fig. 2. Evolution of histamine concentration for white grape varieties during fermentation.

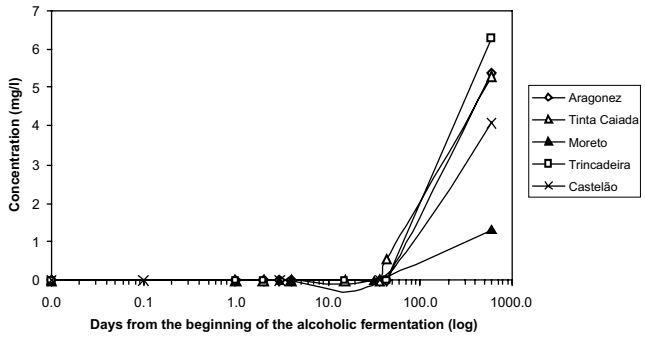

Fig. 3. Evolution of tyramine concentration for red grape varieties during fermentation.

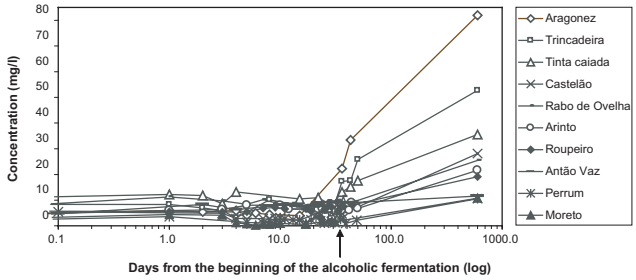

Fig. 5. Total biogenic amine concentration in each grape variety during fermentation. The arrow indicates the time in which the red wine completed malolactic fermentation (33 days on average, maximum 36, minimum 30).

contradiction with the conclusions of Soufleros et al. (1998) and Vidal-Carou, Codony-Salcedo, et al. (1990b), who found significant correlation between the levels of tyramine and histamine during the fermentation process. From the fermentations studied, it seems that tyramine results from malolactic fermentation, since an increase in this amine was only noticed in red wines after this type of fermentation.

The evolution of putrescine in red wines was identical to that of tyramine although not as consistent, since two of the wines analyzed showed no increase of this amine. As shown in Fig. 4, except for one, in which the level of putrescine was exceptionally high after 18 months in storage, the average level of this amine in red wines (15.0 mg/l) was about three times as high as in musts from which they were derived (4.4 mg/l).

High levels of putrescine in wines may be connected to poor hygiene conditions during production (Radler & Fäth, 1991). Nevertheless, those levels may be of difficult definition, due to the wide range of concentrations found in wines, such as 12.5 mg/l mean minimum, and 138 mg/l maximum (Soufleros et al., 1998). Malolactic fermentation appears to be the source of tyramine and putrescine for the red wines studied, as opposed to white wines, where the source relies on the levels initially present in the musts.

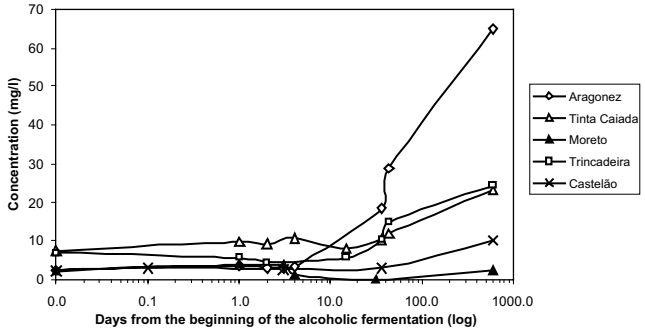

Fig. 4. Evolution of putrescine concentration for red grape varieties during fermentation.

Results for the variation of total biogenic amine levels for individual varieties are presented in Fig. 5. Among those studied, Tinta Caiada, Aragonez and Trincadeira varieties presented the highest levels of total biogenic amines, at the end of fermentation.

It should be emphasized that the varieties with the highest final concentrations of biogenic amines were also those with highest levels of assimilable amino acids. For red wines, the final concentrations of assimilable amino acids were as follows, in decreasing order: Aragonez: 781 mg/l; Trincadeira: 382 mg/l; Tinta Caiada: 354 mg/l; Castelão: 255 mg/l and Moreto: 69 mg/l. For white wines: Rabo de Ovelha: 1367 mg/l; Roupeiro: 1214 mg/l; Arinto: 1057 mg/l; Antão Vaz: 965 mg/l and Perrum: 286 mg/l. For total biogenic amines, concentrations followed the same order for each variety (Fig. 5). In white wines, apart from the change in position between Arinto and Roupeiro, the relationship remained unchanged.

Being essential nutrients for yeast and bacteria, free amino acid levels may have influence on the development of these microorganisms, thus affecting its metabolism and the final profile of fermentation products. In a study where 286 wines were evaluated regarding the histamine content, Ough (1971) found a significant correlation between histamine and total nitrogen levels.

3.2. Influence of variety on assimilable amino acid and amine contents

The influence of variety on levels of free amino acids and amines was evaluated according to levels of these compounds in musts (Table 3) and wines (Table 4) from different grape varieties obtained from the same subregion of Évora. The wines and respective musts from 1998 were used for each grape variety. As seen in Table 3, musts from the white varieties show, in general, average levels of free amino acids higher and more variable than those of the red musts. The white variety Perrum was an exception, having the lowest level of assimilable amino acids of all the varieties studied.

Table 3
Levels (mg/l) of assimilable amino acids, volatile and biogenic amines in musts from different grape varieties in the same sub-region (Évora), and in musts from Trincadeira (red) and Roupeiro (white) varieties, in different sub-regions, in 1998

Parameter	Assimilable amino acids	Volatile amines	Biogenic amines
Variety: white musts			
Roupeiro	1489	37.0	5.9
Perrum	743	20.3	1.8
Rabo de Ovelha	1687	31.4	6.2
Arinto	1607	34.4	4.2
Antão Vaz	1350	26.3	4.2
Mean	1375	29.9	4.5
CV (%)	27	22	39
Variety: red musts			
Castelão	952	15.1	2.0
Tinta Caiada	1254	30.6	10.5
Aragonez	1053	24.1	3.7
Trincadeira	935	27.5	8.4
Moreto	1063	14.1	2.3
Mean	1051	22.3	5.4
CV (%)	12	33	71
Sub-region: musts Roupeiro			
Portalegre	1027	22.5	4.3
Reguengos	852	17.2	4.4
Redondo	1167	25.9	5.4
Vidigueira	1065	28.7	10.6
Évora	1489	37.0	5.9
Mean	1120	26.3	6.1
CV (%)	21	28	42
Sub-region: musts Trincadeira			
Portalegre	1257	34.3	7.7
Reguengos	748	15.3	8.0
Redondo	710	22.7	5.4
Évora	935	27.5	8.4
Borba	711	12.4	2.1
Mean	872	22.4	6.3
CV (%)	27	40	42

CV—coefficient of variation.

Table 4
Levels (mg/l) of assimilable amino acids, volatile and biogenic amines in wines from different grape varieties in the same sub-region (Évora), and in wines from Trincadeira (red) and Roupeiro (white) varieties, in different sub-regions, in 1998

Parameter	Assimilable amino acids	Volatile amines	Biogenic amines
Variety: white wines			
Roupeiro	1214	25.3	19.1
Perrum	286	11.8	10.3
Rabo de Ovelha	1367	23.8	24.8
Arinto	1057	20.3	20.9
Antão Vaz	965	22.5	10.9
Mean	978	20.7	17.2
CV (%)	42	26	37
Variety: red wines			
Castelão	255	22.7	28.1
Tinta Caiada	354	34.8	29.3
Aragonez	781	35.6	82.0
Trincadeira	382	33.3	52.6
Moreto	69	11.5	4.5
Mean	368	27.6	39.3
CV (%)	71	38	74
Sub-region: wines Roupeiro			
Portalegre	513	15.7	12.5
Reguengos	316	16.7	12.0
Redondo	401	20.4	12.4
Vidigueira	610	23.8	14.5
Évora	1214	25.3	19.1
Mean	611	20.4	14.1
CV (%)	58	21	21
Sub-region: wines Trincadeira			
Portalegre	630	43.6	60.0
Reguengos	282	26.9	22.9
Redondo	258	23.6	21.3
Évora	382	33.3	52.6
Borba	101	13.8	8.2
Mean	331	28.2	33.0
CV (%)	59	39	67

CV—coefficient of variation.

Lower levels of volatile and biogenic amines were also found in the musts and wines of this variety.

As to white musts, it appears that variety affected mainly the biogenic amine contents of red musts, as seen in Table 3 by the coefficient of variation values.

With the exception of assimilable amino acids, variety consistently provoked higher variability in all parameters for red musts and wines than for white ones.

In wines (Table 4), fermentation and storage phases seemed to mask the influence of variety, especially on assimilable amino acids content, by increasing the variability among varieties. Coefficients of variation obtained for the different variables in wines are certainly the sum of the variety and fermentation influences. This is particularly important if alcoholic and malolactic fermentations are performed by wild strains of yeast and bacteria. The high coefficient of variation for red wines in respect to assimilable amino acids and biogenic amines is the result of different interactions between variety, yeast and bacteria.

3.3. Influence of sub-region on assimilable amino acid and amine contents

The influence of sub-region was evaluated according to the study of Roupeiro (white) and Trincadeira (red) varieties, cultivated in different Alentejo sub-regions. It must be pointed out that 1998 Roupeiro wines were not sampled in Borba, neither were Trincadeira musts and

wines in the Vidigueira sub-region. It was noticed that the regional factor had a similar effect on musts from Roupeiro and Trincadeira (Table 3).

For red variety Trincadeira, the Borba sub-region was the one that originated the musts less rich in assimilable amino acids and volatile and biogenic amines. The musts from Évora showed the highest levels.

Évora and Vidigueira, in the case of Roupeiro and Évora and Portalegre in the case of Trincadeira, presented the highest levels of biogenic and/or volatile amines (Table 3). Biogenic amine contents in musts were the variables most affected by the region factor, as was the case for variety. In general, the influence of the region in the different wines is identical to that observed for the musts (Table 4). Borba was the source of the Trincadeira wines less rich in assimilable amino acids and biogenic and volatile amines (note that Roupeiro wines from this region were not tested), and Évora and Vidigueira were those which had Roupeiro wines with the highest levels of assimilable amino acids and biogenic and volatile amines, as was the case for their musts. In Trincadeira wines, those from Portalegre showed the highest levels of assimilable nitrogen and volatile and biogenic amines, as happened with the musts.

The coefficient of variation for assimilable amino acid contents was higher for wines than for musts. As seen before for variety, alcoholic (and malolactic) fermentation increased biogenic amine contents variability amongst red wines and decreased it for white ones. This suggests that malolactic fermentation is probably the major source of variability for red wines.

3.4. Influence of vintage on assimilable amino acid and amine contents

There were no remarkable differences in assimilable amino acid and biogenic amine levels of musts or wines from the two years studied for the different varieties (in the same sub-region) and for different sub-regions (for the same varieties).

Volatile amine content (mg/l) was the sole variable for which statistically significant differences were found (at 95% level) between the two years studied, namely for Trincadeira wines (18.8 ± 8.3 in 1997 and 28.2 ± 11.1 in 1998) and white musts (29.9 ± 6.7 in 1998 and 20.5 ± 3.9 in 1999).

These results agree in part with those published by Sass-Kiss et al. (2000), who showed that vintage and the technology employed were the main factors affecting the levels of amines in the studied wines, both qualitatively and quantitatively. Bearing in mind that the concentrations of assimilable amino acids did not vary significantly between the two years, either in the different sub-regions or for the different varieties, it appears that the discrepancies in amine levels observed may be due to other factors, such as microbiology. This being the case, the influence of the various populations of yeast and bacteria, naturally selected each year, might be controlled through the inoculation of appropriate strains.

4. Conclusions

A large number of samples (209) comprising wines and musts from different varieties, Alentejo sub-regions and vintages were studied. These included the samples collected during 10 microvinifications.

For the majority of the samples, the amines cadaverine, tryptamine, β-phenylethylamine and isoamylamine were found to be below the limit of detection of the method used.

Throughout alcoholic or malolactic fermentation (that occurred in all red wines), no significant increase was noticed in the levels of total volatile amines. In relation to histamine, its concentration did not vary greatly during alcoholic fermentation, being maintained close to the values initially found in musts (average 1.2 mg/l for red musts). While higher histamine levels were found in the storage period only, an increase in the tyramine concentration was confirmed in all 5 vinifications immediately after malolactic fermentation.

In the red wines studied, malolactic fermentation seems to be the main source of tyramine and putrescine.

Grape variety, region of production and vintage can affect free amino acid and amine contents in both musts and wines, although alcoholic and malolactic fermentations can overcome that influence.

The varieties with the highest final concentrations of biogenic amines were also the most prominent in assimilable amino acids levels.

Differences among musts from different varieties were mainly found in the biogenic amine contents of red musts. However, volatile amine and assimilable amino acid contents were also affected by the same parameter, although the latter were less affected in red musts than in white musts.

Biogenic amine content in musts was the variable most affected by the sub-region factor.

Studying the influence of vintage, it was shown that the volatile amines were the group of compounds with the most pronounced differences.

In general, low levels of biogenic amines were found in the musts or wines studied, especially if compared with other foodstuffs, where biogenic amines can occur in much higher concentrations.

Acknowledgements

The authors wish to thank to CVRA for their support and to PRAXIS XXI for scholarship PRAXIS/BD/3261/96.

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