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Relationship Between Biogenic Amines and Free Amino Acid Contents of Wines and Musts from Alentejo (Portugal)

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The concentration of biogenic amines and free amino acids was studied in 102 Portuguese wines and 18 musts from Alentejo demarcated (D.O.C.) regions. Most wines were commercial, except for 38 monovarietals obtained by micro vinification. Musts from the varieties used to produce the latter wines were also studied. Both biogenic amines and free amino acids were analyzed by HPLC using fluorescence detection for their o-phthalaldehyde/fluorenylmethyl chloroformate (OPA/FMOC) derivatives. The most significant amines (average 10.8 mg/L for histamine+tyramine in red, and 7.4 mg/L for white wines) were found to be present at low levels and, although no important relationship between each individual biogenic amine could be obtained, the total amine content depends significantly on the assimilable amino acid content in wine.

Key Words: Amines; Amino acids; Wines; Musts.

INTRODUCTION

The demands of consumers regarding the characteristics of food and beverages are nowadays very specific in relation to certain aspects, and wine is no exception. Consumers have increasingly been demanding new products, which can carry benefits and/or less damage to human health. These facts continuously

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modify the demands on food authenticity, quality and safety. An important group of compounds that exists normally in wines, free amino acids, are often studied with the above mentioned purposes.

Free amino acid contents directly affect wine quality, because they interfere with the levels of some trace compounds which enhance that quality, such as aroma compounds[1] or which have physiological significance, such as ethyl carbamate,[2–5] or which can even be related to wine authenticity.[6–8] Besides this, free amino acids are precursors of biogenic amines, other trace compounds important to human health existing in wines. These, which although usually present in small quantities, interfere with the human metabolism, (e.g. vasoactive or psychoactive properties), thus justifying research based on their origin and presence in wines.

The biogenic amines with adverse effects on human health that are normally found in wines are the following, together with their metabolic amino acid precursors:

i) aromatic and heterocyclic amines: histamine (derived from histidine), tyramine (from tyrosine), β-phenylethylamine (from phenylalanine) and tryptamine (from tryptophane);

ii) aliphatic di-, tri- and poli-amines: putrescine (derived from arginine and ornithine), cadaverine (from lysine), agmatine (from arginine) and spermidine and spermine (from putrescine).

Other amines, that have no negative effects on human health described, but that might act synergistically with biogenic amines are the aliphatic volatile amines: ethylamine, methylamine, isoamylamine and ethanolamine. These need to be identified and quantified in wines, in order to prevent the alteration of sensorial properties.

Currently, more than 25 different amines have been identified in wines.[9] However, neither the origins nor the conditions that drive the formation of biogenic amines in wines are yet completely understood. Studies have been reported relating biogenic amines content with SO_2,[10–12] malolactic fermentation,[11–13] pH,[14] precursor amino acids,[15] or total nitrogen content.[16] In some cases, those studies suggest dependence between amines content and one or more of those factors. However, there are several works pointing apparently to different conclusions.

Concerning the origin of biogenic amines, some authors refer to malolactic fermentation as the step in which the bulk of these amines, including histamine and tyramine, are formed.[11,17] In an important work, Soufleros et al.[18] reported a significant increase in biogenic amines content during and after the spontaneous malolactic fermentation. They also found a relationship between the content of free amino acids and compounds related with wine spoilage and the concentration of biogenic amines in the wines studied.

However, others find no relationship between malolactic fermentation and biogenic amines formation, indicating the alcoholic fermentation as the main source of these amines instead,[19] and in fact stating a decrease in some amines such as histamine, putrescine and cadaverine during malolactic fermentation. As an example, Vidal-Carou et al.[20] observed a decrease in the content of histamine and tyramine in some wines even after its spoilage at various temperatures. Furthermore, there is not even a consensus on the identity of the microorganisms responsible for the main production of biogenic amines during the winemaking process.

It is clear that some factors seem to correlate very well with amines formation in some conditions, but the relationship is lost in others. Nevertheless, because some of the above mentioned parameters can be controlled up to a certain degree, it is important to obtain data about the wines from each region in order to assure the highest quality in each case. This will also contribute to a larger data set that could help in a better understanding of the process of amine formation, and the establishment of reasonable limits for the contents of biogenic amines.

This work aimed to study the contents of biogenic amines and free amino acids in wines from the Alentejo region (Portugal), and to seek relationships between these parameters and free amino acids levels.

MATERIALS AND METHODS

Samples

A first screening set of 38 samples (SET I) was studied comprising monovarietal wines from 1997 (20 samples) and 1998 (18 samples). These wines were produced by microvinification with the main cultivars from the Alentejo region and sub-regions Arinto, Perrum, Antão Vaz, Rabo de Ovelha and Roupeiro for white wines, and Aragonez, Moreto, Castelão, Tinta Caiada and Trincadeira for red wines. Trincadeira and Roupeiro were from Évora, Portalegre, Borba, Reguengos, Redondo and Vidigueira sub-regions. All the other cultivars are from Évora. A second set, SET II, of 64 wines (30 white and 34 red) was studied. These were all commercial 1997 white and red wines from the Alentejo region and sub-regions Portalegre, Borba, Redondo, Reguengos, Vidigueira, Évora, Moura and Granja. SET III included the musts of the varieties referred above, that had been used to produce the 1998 wines in SET I. Musts were sampled in the beginning and at the end of fermentation. The volume obtained in each microvinification was about 40 L for red wine, without temperature control, and 20 L for white wine, fermented at 17 °C. Samples were immediately frozen and kept at −15°C until analysis.

Analytical Methods

Free amino acids and biogenic amines quantification was performed according to a previously developed HPLC method,[21] whose principle is a previous derivatization of samples with *o*-phthalaldehyde and 9-fluorenylmethyl chloroformate and subsequent separation by HPLC with fluorescence detection. The total runtime is 138 minutes, and it allows the identification and quantification of 21 free amino acids, 2 intermediates of the urea cycle and 10 biogenic amines in musts and wines. Average detection limits based on the calibration curves were 0.58 mg/L on average for amino acids (except for arginine which was −2.95 mg/L), 0.28 mg/L for amines, and 27.3 mg/L for proline. 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. Average accuracy, calculated by the standard addition method, was 99.8% (coefficient of variation 11.1%; $n = 6$), with minimum and maximum recovery obtained for isoamylamine (81%) and histamine (138%), respectively.

All other parameters, namely °Brix, free and total sulfur dioxide, total and volatile acidity, alcohol content and pH, were determined in compliance with the Office International de la Vigne et du Vin (O.I.V.) methods.[22]

Waste Disposal

In this work, whenever possible, the waste produced was recovered, 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.[21]

Statistical Analysis

Regressions and correlations were carried out using Statgraphics® Plus v. Windows 1.4 1995 (Manugistics, Maryland, USA).

RESULTS AND DISCUSSION

The results from the 64 wines in SET II were used to establish relationships between amine content and free amino acids contents and were supported by SET I samples. Data from SET III samples allowed the establishment of relationships between the initial content of free amino acids in musts, and the amine content in the resulting wines.

Chemical Characterization of the Samples

For monovarietal red and white wines (SET I), free sulphur dioxide levels (mg/L) were (mean ± standard deviation) 23.5 ± 11.1 and 36.4 ± 8.0, respectively. Total sulphur dioxide levels (mg/L) were 84.2 ± 21.2 for red and 110.9 ± 13.1 for white wines. Alcohol content (% v/v) was similar (12.7 ± 1.0) for both wine types, as were reducing sugars levels (2.39 ± 1.13) and pH (3.78 ± 0.26). Red wines had higher volatile acidity (g/L) (0.52 ± 0.12) than white ones (0.24 ± 0.13), which could be due to the occurrence of malolactic fermentation in the former. Titrable acidity (g/L) was 4.18 ± 0.53 for red and 4.33 ± 0.56 for white wines.

As to commercial wines (SET II), the parameters taken into account showed a similar relation between red and white ones. Volatile acidity (g/L) was 0.59 ± 0.10 for red and 0.39 ± 0.11 for white and free sulphur dioxide levels (mg/L) were 4.7 ± 4.2 for red and 8.0 ± 6.2 for white. Total sulphur dioxide (mg/L) was 39.4 ± 22.1 for red and 67.2 ± 27.1 for white wines. As before, levels for alcohol content, reducing sugars and pH were similar for both types of wines, with values of 12.4 ± 0.7 (%v/v), 2.43 ± 0.74 (g/L) and 3.45 ± 0.20, respectively. The main difference between SET II and SET I was the lower sulphur dioxide content of the former.

The reducing sugar content of both red and white musts (SET III) was similar, as were titrable acidity and pH. In general, musts (mean ± standard deviation) had initial °Brix levels of 21.4 ± 1.5, reducing sugars of 209 ± 16 (g/L), titrable acidity of 4.8 ± 0.9 (g/L), and pH of 3.62 ± 0.22, with all parameters considered within the expected range.

Free Amino Acids and Biogenic Amines Content

In terms of free amino acids and amines content, the results of the analysis of wines (SET I and II) and musts (SET III) are expressed in Tables 1 and 2, respectively. For this study, total aminogenic amino acids were considered as the sum of histidine, arginine, tyrosine, phenylalanine and lysine, precursors of the corresponding (biogenic) histamine, putrescine, tiramine, β-phenylethylamine and cadaverine amines, and the sum of all amino acids but proline yielded the total assimilable amino acids.

In both SET I and SET II, white wines had slightly higher values of total amino acid contents (average 1601 and 1439 mg/L, respectively) than red wines (1570 and 1392 mg/L, respectively). A similar trend was noticed for the average content of assimilable amino acids, higher in white wines (635 mg/L) than in red wines (320 mg/L) in SET I and in SET II (555 mg/L for white and 363 mg/L for red, respectively) wines. These differences for total and assimilable amino acids mainly reflected a higher content of arginine in white wines than in red wines. Proline represented, on average, a lower percentage of total amino acids

Table 1: Free amino acid and amine contents (mg/L) for SET I and SET II samples.

	SET I						SET II					
	White wines n = 19			Red wines n = 19			White wines n = 19			Red wines n = 19		
Variable	Average	Max.	Min.	Average	Max.	Min.	Average	Max.	Min.	Average	Max.	Min.
Asp	26.2	42.8	11.3	20.9	36.1	3.4	27.1	50.0	9.9	20.6	57.0	1.3
Glu	27.7	46.1	13.7	23.7	45.7	6.1	28.0	71.9	8.8	29.0	134.3	2.7
Asn	38.2	73.0	12.5	15.2	57.5	0.7	21.0	149.1	1.7	12.3	44.9	2.1
Ser	25.6	54.8	10.9	20.3	46.9	3.5	17.5	34.1	5.0	15.2	54.5	nd
Gln	2.8	7.8	nd	1.5	18.2	nd	0.3	1.3	0.0	0.3	3.5	nd
His	35.8	108.5	6.5	14.1	55.9	nd	14.6	34.9	0.0	6.2	19.5	nd
Gly	27.1	48.8	10.1	18.2	44.3	nd	16.0	30.6	8.5	19.5	54.4	4.7
Thr	21.7	74.2	7.4	18.0	47.4	6.0	12.2	24.9	4.9	12.0	48.1	2.7
Cit	10.3	31.1	3.9	6.5	21.3	2.1	6.5	23.9	nd	6.1	22.9	nd
Arg	126.3	336.1	15.4	29.4	183.2	nd	117.4	497.7	18.0	48.7	240.2	1.5
Ala	87.0	239.6	10.9	31.7	152.2	nd	30.8	63.1	7.1	32.9	128.2	3.2
GABA	42.3	99.4	6.9	18.4	85.4	2.7	30.5	183.5	2.1	18.4	80.1	2.5
Tyr	20.3	61.6	6.5	7.6	20.4	0.0	17.3	44.1	7.4	9.8	48.6	nd
Val	18.6	75.9	5.4	11.4	21.6	5.1	11.9	28.1	nd	11.0	38.2	1.7
Met	5.3	11.9	0.6	2.7	9.2	nd	21.3	98.3	2.4	18.1	95.7	0.8
Trp	9.4	21.6	3.4	6.0	13.4	3.4	3.9	8.0	nd	3.8	7.6	nd
Phe	11.1	42.5	3.8	5.0	12.4	nd	55.6	121.0	19.9	33.0	88.4	9.1
Ile	10.7	26.5	4.2	7.1	12.9	2.2	10.8	25.5	2.7	8.9	31.0	1.5
Leu	31.3	58.9	0.0	26.6	46.8	6.0	27.8	52.0	nd	14.5	55.0	1.8
Orn	16.9	53.0	1.9	0.8	3.3	nd	9.7	44.2	nd	10.8	71.4	nd
Lys	40.1	81.6	17.2	35.3	60.1	7.8	76.8	165.5	20.0	41.8	93.4	6.6
Pro	966	1207	327	1250	1591	950	885	1329	463	1028	1631	349

(Continued on next page)

Table 1:

Ass. a.a.	635	1367	189	320	781	69.4	555	1278	194.7	363	1266	65.2
Total a.a.	1601	2501	613	1570	2158	1096	1439	2069	843.1	1392	2008	669.0
ETA	17.4	22.3	11.1	19.9	34.3	11.1	17.4	37.8	nd	29.5	58.6	5.7
HISTA	10.2	18.2	5.1	9.9	21.2	6.1	1.8	5.2	nd	4.2	12.0	nd
METILA	0.7	2.9	0.2	0.4	3.2	nd	2.2	5.9	0.6	2.8	7.7	0.7
ETILA	1.7	3.2	<0.24	3.9	8.7	0.4	3.3	8.3	nd	6.1	11.8	nd
TIRA	0.3	0.4	nd	2.4	7.5	nd	2.5	5.4	1.0	5.0	15.0	1.3
PUT	4.3	7.6	1.0	14.6	65.0	1.3	9.3	26.1	2.3	18.7	40.3	4.1
CAD	0.6	1.3	<0.17	1.2	2.2	0.6	4.0	11.2	nd	3.7	11.8	1.3
FEN	—	<0.47	nd	—	<0.47	nd	—	—	—	—	—	—
TRY	0.4	0.6	<0.35	<0.35	0.7	nd	—	—	—	—	—	—
ISO	0.3	0.8	nd	0.2	0.6	nd	2.0	4.5	nd	2.4	3.4	0.7

Legend: Asp—aspartate; Glu—glutamate; Asn—asparagine; Ser—serine; Gln—glutamine; His—histidine; Gly—glycine; Thr—threonine; Cit—citrulline; Arg—arginine; GABA—γ-aminobutiric acid; Tyr—tyrosine; Val—valine; Met—methionine; Trp—tryptophane; Phe—phenylalanine; Ile—isoleucine; Leu—leucine; Orn—ornithine; Lys—lysine; Pro—proline; Ass. a.a.-assimilable amino acids (all amino acids except proline); Total a.a.-total amino acids; ETA—ethanolamine; HISTA—histamine; METILA—methylamine; ETILA—ethylamine; TIRA—tyramine; PUT—putrescine; CAD—cadaverine; FEN—β-phenylethylamine; TRY—tryptamine; ISO—isoamylamine, nd—not detected.

Table 2: Free amino acid and amine contents (mg/L) for SET III must samples.

	White musts n = 9			Red musts n = 9			White musts n = 9			Red musts n = 9		
Variable	**Average**	**Max.**	**Min.**	**Average**	**Max.**	**Min.**	**Variable**	**Average**	**Max.**	**Min.**	**Average**	**Max.**	**Min.**
Asp	46.5	90.4	33.6	30.8	41.3	20.8	Ile	46.7	106.5	12.3	24.1	45.9	12.2
Glu	44.0	64.9	31.9	37.0	50.6	28.9	Leu	61.5	107.0	20.6	36.1	56.6	14.8
Asn	28.8	53.0	6.7	21.0	68.6	7.1	Orn	2.6	13.5	0.0	0.8	3.6	0.0
Ser	52.2	70.8	41.0	53.1	69.4	40.8	Lys	16.5	25.3	7.2	10.1	15.5	3.3
Gln	107.5	124.8	94.3	95.3	117.9	69.1	Pro	910.5	1241	34.0	968.9	1296	638.0
His	101.0	157.8	26.8	50.1	97.2	11.7	Ass. a.a.	1221	1687	743.4	964.8	1257	710.5
Gly	10.7	33.5	2.4	4.3	9.3	0.0	Total a.a.	2131	2849	777.4	1934	2398	1349
Thr	84.6	106.7	56.6	70.9	86.7	53.0	ETA	18.1	26.2	12.1	15.4	25.0	7.8
Cit	13.3	25.2	6.2	6.2	18.7	0.0	HISTA	nd	nd	nd	1.2	2.4	nd
Arg	309.7	368.9	239.8	312.2	435.1	231.7	METILA	6.7	13.2	2.6	4.1	6.7	2.1
Ala	40.6	119.5	0.1	71.5	141.6	17.9	ETILA	2.3	4.9	1.0	2.3	6.0	nd
GABA	74.1	118.1	29.9	47.3	110.3	20.2	TIRA	0.1	0.4	nd	nd	nd	nd
Tyr	36.7	65.2	13.9	22.1	45.9	4.7	PUT	4.3	6.7	0.8	4.5	7.4	1.2
Val	77.0	138.6	11.4	45.2	87.1	21.8	CAD	nd	nd	nd	0.5	1.3	nd
Met	11.2	24.5	1.7	7.8	18.5	0.7	FEN	<0.47	0.9	nd	<0.47	0.5	nd
Trp	10.9	17.8	5.5	12.4	21.5	4.8	TRY	0.9	3.6	nd	<0.35	0.7	nd
Phe	44.7	85.5	9.3	6.5	15.0	0.8	ISO	nd	nd	nd	nd	nd	nd

Legend: Asp—aspartate; Glu—glutamate; Asn—asparagine; Ser—serine; Gln—glutamine; His—histidine; Gly—glycine; Thr—threonine; Cit—citrulline; Arg—arginine; GABA—γ-aminobutiric acid; Tyr—tyrosine; Val—valine; Met—methionine; Trp—tryptophane; Phe—phenylalanine; Ile—isoleucine; Leu—leucine; Orn—ornithine; Lys—lysine; Pro—proline; Ass. a.a.-assimilable amino acids (all amino acids except proline); Total a.a.-total amino acids; ETA—ethanolamine; HISTA—histamine; METILA—methylamine; ETILA—ethylamine; TIRA—tyramine; PUT—putrescine; CAD—cadaverine; FEN—β-phenylethylamine; TRY—tryptamine; ISO—isoamylamine, nd—not detected.

in white wines for both sets (60% in SET I and 62% in SET II) than in red wines (80% in SET I and 74% in SET II).

As expected, both total and assimilable amino acids are higher in musts (SET III, Table 2) than in wines. There is a general decrease of free amino acid during fermentation, once free amino acids are essential nutrients for yeast growth. The average content in assimilable amino acids in red and white musts was 964.8 mg/L and 1221 mg/L, respectively. However, arginine content is not responsible for this difference in musts as it was for wines. Instead, it is due to an overall higher concentration of free amino acids in white musts. The concentration of glutamine in musts (SET III) is dramatically decreased by the winemaking process in both red and white wines (Tables 1 and 2). At the same time, it seems that there is a release of glycine and lysine during the fermentation of white and red musts and a release of ornithine during the fermentation of white musts. The proline content in musts is consistent with the slightly higher content of proline in red over white wines, and similar in musts and wines, which agrees with the fact that proline is not usually metabolized during must fermentation.

The values obtained for the concentrations of free amino acids in these wines and musts are comparable in magnitude to others from previous works,[6,23] as are those found for citrulline (average 10.3 mg/L for white wines and 6.5 mg/L for red wines of SET I, 6.5 mg/L for white and 6.1 mg/L for red wines of SET II) and ornithine (average 16.9 mg/L for white wines and 0.8 mg/L for red wines in SET I, 9.7 mg/L for white and 10.8 mg/L for red wines in SET II.)[24,25] These two particular compounds have pronounced enological interest once they are intermediaries in the urea cycle. Urea and citrulline can directly react with ethanol to produce ethyl carbamate, a compound with carcinogenic properties.

The general amines content in the musts from SET III is lower than in the resulting wines, or in the other wines studied (Tables 1 and 2). However, the ethanolamine level in the analyzed musts is similar to its concentration in wines, and this may indicate that the grapes were the main source of this amine in these wines. Ough et al.[2] identified the presence of several volatile amines such as ethylamine, methylamine and isoamylamine, among others, for the first time in grapes. Methylamine concentration seems to decrease during the fermentation process, because the average concentration of this amine is higher in musts than in wines. While ethanolamine is the most prominent amine found in the musts studied (average 50% of the total amines), isoamylamine was not found, and white musts contained more histamine on average (5.9 mg/L) than red musts (2.1 mg/L). For wines (Table 1), ethanolamine represented, on average, 42% (SET I), and 41% (SET II) of the total amines found.

The mean value for the biogenic amines histamine+tyramine content was 9.9 mg/L for red wines, and 10.2 mg/L for white wines in SET I and 4.2 mg/L

for red wines and 1.8 mg/L for white in SET II. Putrescine represented an important fraction of the total content in amines in both wine sets. Phenylethylamine and tryptamine were not quantified in wines from SET II, due to analytical problems.

The results obtained for the studied wines are comparable to those obtained by other authors,[20,26] while the results referring to the histamine content in musts are slightly higher than those normally reported in literature. Usually, histamine is not present in grape juice in amounts higher than a few tens of milligrams per liter.[12,27]

Relation between Amines and Free Amino Acids

Due to the close relation between amines and free amino acids, the influence of free amino acid contents in wines and musts on amine content was studied for monovarietal (SET I) and commercial (SET II) wines. Monovarietal wines were used to seek relationships between amine and free amino acid contents in wines. Commercial wines tested were used to evaluate if results for the first group of samples (obtained from micro-vinifications), could be extended to the commercial ones a much more heterogeneous group. Results from 1998 must samples allowed the establishment of relationships between the content in amines in wines with the initial values of the musts in terms of assimilable amino acids, and aminogenic amino acids.

Relationship between Amines and Free Amino Acids in SET I Wines

The results of the correlations obtained for the wines of the SET I are expressed in Table 3. The highest correlation coefficients were obtained between total amines and assimilable amino acids ($R = 0.913$ for red and $R = 0.764$ for white wines). This parameter seems to be the most important regarding amine formation in the wines studied (Fig. 1). Consistently, correlation coefficients found for red wines were higher than those found for white wines. Availability of biogenic amines precursors in wines is probably less important than the availability of assimilable amino acids. In red and white wines, biogenic amines were better correlated with assimilable than with aminogenic amino acids. Considering the low levels of biogenic amines and the levels of aminogenic amino acids usually found in wines, these results supposedly indicate that lowering the concentration of assimilable amino acids in wines, instead of the concentration of aminogenic amino acids, will reduce the biogenic amine content effectively.

At least for lactic acid bacteria, biogenic amine production may constitute a mechanism of metabolic energy generation. The generation of a proton motive force by decarboxilation of histidine and antiport excretion

Table 3: Correlation coefficients and significance of the linear regression analysis for SET I and SET II wines.

RED / WHITE	Total amines		Biogenic amines		HISTA+TIRA	
	R	S	R	S	R	S
			SET I			
Ass. a.a.	0.913	<0.001	0.897	<0.001	0.519	<0.05
	0.764	<0.001	0.696	<0.001	0.680	<0.01
Aminogenic amino acids	0.819	<0.001	0.852	<0.001	0.455	ns
	0.752	<0.001	0.649	<0.01	0.588	<0.01
			SET II			
Ass. a.a.	0.438	<0.01	0.446	<0.01	0.14	ns
	0.666	<0.001	0.627	<0.001	0.528	<0.01
Aminogenic amino acids	0.379	<0.05	0.379	<0.05	—	ns
	0.553	<0.01	0.424	<0.05		

Legend: Ass. a.a.-assimilable amino acids (all amino acids except proline); Aminogenic amino acids: His, Arg, Tyr, Phe, Lys; R—correlation coefficient; S—significance; ns—not significant

of histamine to the medium, was already demonstrated for *Lactobacillus* buchneri.[28] In addition, other authors state that histamine accumulation is a result of bacterial growth in poor media,[27] since histamine production was observed only under non-proliferation conditions, in spite of optimal growth

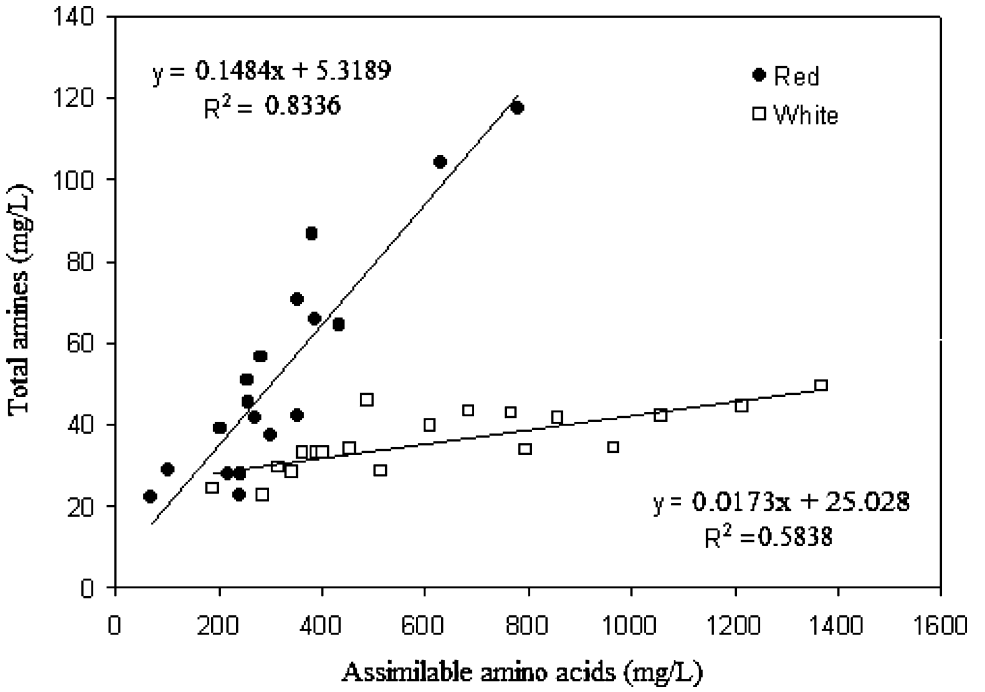

Figure 1: Relationship between total amine and assimilable amino acid content in SET I wines.

conditions. In this case, the decarboxilase system should be inducible instead of constitutive. The expression of such systems would occur as a function of the nutritional and energetic constitution of the medium and depending on the growth phase of the microorganisms.[29] Thus, it is possible that large quantities of assimilable amino acids in the medium could induce great microbial growth not sustainable in latter stages, by completely removing other nutrients. Consequently, those microorganisms could produce amines during a non-proliferation phase as a process of metabolic energy gathering. Under such conditions, the availability of each amine precursor is determinant for the overall accumulation, once amino acid decarboxilation and amine excretion appear to be stoichiometric processes.[28] This could also explain the correlation between histamine and total nitrogen levels of California wines found by Ough[16] and justify why amine accumulation occurs sometimes at the end or after the fermentation processes.

Relationship between Amines and Free Amino Acids in SET II Wines

The results of the correlations between several parameters and the significance of the linear regression obtained for wines of the SET II are expressed in Table 3. These results show that the total amine content in the wines studied significantly depends on the assimilable amino acid levels ($R = 0.666$ for white wines and $R = 0.438$ for red), with a probability of at least 99%. White wines present a higher correlation coefficient (and greater statistical significance of $p < 0.001$) than red wines, contrary to what happens in SET I, possibly due to a greater heterogeneity in the red wines group. Nevertheless, with such heterogeneity clearly present between all wines studied, an average correlation coefficient of 0.552 (between red and white wines) seems relevant. As expected, these values are lower than in SET I, once several technological factors were "normalized" in the production of these wines. In the same way, total amine content has a significant relationship with aminogenic amino acids, although lower than in the former case.

Total amines are expected to be better correlated with assimilable amino acids than with aminogenic amino acids, once the latter group does not include the precursors of the volatile amines. It also seems that the availability of biogenic amines precursors in these wines is not as important as the availability of assimilable amino acids. In red and white wines, biogenic amines were better correlated with assimilable amino acids (average $R = 0.536$) than with the aminogenic only (average $R = 0.402$) (Table 3).

Bauza et al.[15] found significant correlation between putrescine and its precursors in wines, in opposition to β-phenylethylamine, tyramine or histamine and its precursors. No mentionable relationship was detected in this work between each individual biogenic amine and its precursor(s), in SET II.

With respect to histamine and tyramine content, again it depends more on the content of assimilable amino acids in white wines than in red wines. In the latter, no significant relationship was reached.

No mentionable relationship was detected in this work between each individual biogenic amine and its precursor(s) in both SET I and SET II wines. However, if any relation exists, it should be clearer between the level of each amine in wines and the initial content of its precursor in the must prior to fermentation. This study was feasible using the 1998 musts (SET III samples).

Relationship between Amines in Wines and Free Amino Acids in Musts prior to Fermentation–SET III Samples

The analysis of the SET III samples combined with the 1998 SET I wines allowed the correlation of amines content in the wines with the initial values of the musts in terms of assimilable and aminogenic amino acids. Table 4 shows the results of the linear regression analysis using these parameters. Linear regression analysis was performed using amine content in wines as the dependent variable. The first important result is that even with a small set of samples (9 red and 9 white wines), which caused some linear regression analysis to be not statistically significant, the correlation reached between some parameters can be considered very significant. This was clear for white wines, in which the correlation values obtained between the content of total and biogenic amines in wines and the assimilable and aminogenic amino acids in musts were higher than those found for the same parameters in the wines (Table 3).

Linear regression analysis was also performed between the initial content of amine and precursor amino acids in musts and amines in wines. The most important relationship was found between the contents of tyramine and the

Table 4: Correlation coefficients and significance of the linear regression analysis—results of the SET III samples combined with SET I wines from 1998.

	Total amines (wines)		Biogenic amines (wines)		HISTA+TIRA (wines)	
RED **WHITE**	**R**	**S**	**R**	**S**	**R**	**S**
Ass. a.a. (musts)	0.519	ns	0.472	ns	0.604	ns
	0.893	<0.01	0.845	<0.01	0.663	ns
Aminogenic amino acids (musts)	0.388	ns	—	ns	—	ns
	0.864	<0.01	0.782	<0.05	0.724	<0.05

Ass. a.a. = assimilable amino acids (all amino acids except proline).
Aminogenic amino acids = His, Arg, Tyr, Phe, Lys.
R = correlation coefficient.
S = significance.
ns = not significant at 95% level.

precursor amino acid, tyrosine, in red musts and wines ($R = 0.811$; $S < 0.01$). In white wines this relationship was not significant. Furthermore, histamine formation in red wines was not significant at the 95% confidence level ($R = 0.329$), whereas for white wines, histamine could be correlated with the initial histidine content of musts, with 95% of probability ($R = 0.672$). The content on β-phenylethylamine for all wines, also related to the content of its amino acid precursor, phenylalanine, in musts ($R = 0.604$; $S < 0.01$).

In accordance to the previous data obtained for SET I and SET II, the results of the analysis of SET III samples show that the relationship between the amine content in wines is closely linked with the initial content of assimilable amino acids in musts. However, the relationship between the level of each individual amine and its precursor in musts is not clear because amines seem to be affected differently by this parameter. Hence, it is necessary to further investigate the conditions that affect amine formation during fermentations. Nevertheless, these results are technologically important, as it is much easier to control the total amount of assimilable amino acids than the level of each individual amino acid in musts.

The higher biogenic amine content reported here and usually found in red wines can eventually be explained by the occurrence of malolactic fermentation, where assimilable amino acids are needed.[30,31] However, malolactic fermentation normally does not occur in white wines from Alentejo. In this case, it is possible that assimilable amino acids availability during and after alcoholic fermentation for yeast and other fermenting microorganisms could affect white wine content in biogenic amines.

CONCLUSIONS

Biogenic amines are present in wines from the Alentejo in relatively low quantities. The mean average levels of histamine+tyramine are 10.8 mg/L for red wines and 7.4 mg/L for white (from a total of 102 wines). Analyzed musts also contained detectable quantities of biogenic amines; The average content of hystamine+tyramine in 18 samples was 4 mg/L. Concerning the total content of the biogenic amines in wines, it was found that it was better correlated with the total assimilable amino acids (expressed as the total content of free amino acids except proline) than with each aminogenic amino acid. These results suggest that, avoiding high concentrations of assimilable amino acids in musts, which depend, among other factors, on the nitrogen fertilization applied to the vine, could efficiently reduce the content of biogenic amines in wines.

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