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Histamine and Tyramine in Spanish Wines: Relationships with Total Sulfur Dioxide Level, Volatile Acidity and Malo-lactic Fermentation Intensity*

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ABSTRACT

Histamine and tyramine contents in 226 and 186 Spanish wine samples, respectively, have been studied employing spectrofluorometric methods. The average content of histamine in red wines (4·07 mg/liter) proves to be higher than in white wines (0·81 mg/liter) and rosé wines (0·86 mg/liter). The average content of tyramine was also higher in red wines (3·03 mg/liter) than in white (1·49 mg/liter) and rosé ones (1·66 mg/liter). Relationships are studied between the contents of these amines and that of total sulfur dioxide and volatile acidity, as well as with the intensity of malo-lactic fermentation. Our results, in general, show that (a) the highest levels of both amines occur in red wines with low concentrations of total sulfur dioxide, (b) white and rosé wines with high volatile acidity show higher levels of these amines than wines with low volatile acidity, and (c) in all wines highest levels of amines correspond to wines with a stronger malo-lactic fermentation.

INTRODUCTION

Biogenic amines in food proceed from microbial decarboxylation of their precursor amino acids: histidine and tyrosine for histamine and tyramine, respectively. It has been pointed out that this transformation is not direct, but implies more complex biochemical processes (Lafon-Lafourcade, 1975;

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Bravo *et al.*, 1983; Buteau *et al.*, 1984; Somavilla *et al.*, 1986). Some authors consider that formation of biogenic amines in wines is due to alcoholic fermentation yeasts (Quevauviller & Mazière, 1969; Zappavigna *et al.*, 1974; Buteau *et al.*, 1984). On the other hand, most works attribute that formation to malo-lactic fermentation (MLF) bacteria (Marquardt & Werringloer, 1965; Mayer & Pause, 1971; Pechanek *et al.*, 1980). Finally, some authors attribute amine production to contaminating microorganisms; for example, enteric bacteria (Steiner & Laezlinger, 1978; Buteau *et al.*, 1984).

Formation of these amines in wines has been related with lack of hygiene during the wine-making process. Cerutti and Remondi (1972) indicate that 'wine manufactured under optimum conditions from a hygienic viewpoint should practically have no amines'. Likewise, Zappavigna *et al.* (1974) point out that technological conditions of the wine-making process and the quality of raw materials employed have a definite influence on the intensity of amine biogenesis. On that basis, histamine has been proposed as an indicator of defective manufacturing (Battaglia & Fröhlich, 1978) or as a quality parameter of wines (Coppini *et al.*, 1973; Iñigo & Bravo, 1980). Formation of amines in wines does not exclusively depend on the presence of certain microorganisms but also depends on some other factors: (a) content of precursor amino acids in must (Cerutti *et al.*, 1978; Eitenmiller *et al.*, 1978); (b) duration of initial fermentation phase (Burdaspal *et al.*, 1979); (c) level of sulfur dioxide (Rivas *et al.*, 1983); (d) pH (Cerutti & Remondi, 1972); (e) wine container and the period of time the wine remains in it (Gonzalez *et al.*, 1977); (f) contact time of must with grape skin (Ough, 1971; Iñigo & Bravo, 1980).

Biogenic amines are also interesting in food toxicology. Direct toxic effects include food intoxication after consumption of products with high content of histamine (Doeglas *et al.*, 1967; Merson *et al.*, 1974). However, intoxications like these have not been described for wines. Tyramine is related to food migraines (Trethewie & Khaled, 1972; Forsythe & Redmond, 1974). Among indirect toxic effects, the best known is the interaction between biogenic amines and monoamine-oxidase inhibitor drugs (Blackwell & Mabbit, 1965; Asatoor *et al.*, 1973; Ponto *et al.*, 1977).

There has also been a report of possible interaction between histamine and tyramine with other amines (cadaverine, putrescine, spermine and spermidine). This implies a potentiation of amine toxicity (Hui & Taylor, 1983). This effect must be also considered in wines, since various works have shown the presence of interacting amines in them (Schneyder, 1973; Buteau *et al.*, 1984). Likewise, a potentiation of amine effects has been described for the combined presence of ethanol and acetaldehyde, as both substances are inhibitors of monoamine-oxidase (Schneyder, 1973).

In this work, histamine and tyramine contents have been determined in a large number of Spanish wines in order to have data to evaluate eventual technological and toxicological implications of their presence. A study has also been carried out to observe possible relationships between amine contents and total sulfur dioxide level, volatile acidity and the MLF intensity.

METHODS

Histamine has been determined using a spectrofluorometric method developed in our laboratory (Vidal-Carou *et al.*, 1989) which consists of the alkalinization of sample and extraction with *n*-butanol, transference of histamine to hydrochloric acid, formation of a fluorescent complex with *o*-phthalaldehyde and spectrofluorometric reading at 340/430 nm.

Tyramine determination was carried out by the method of Rivas *et al.* (1979). This method consists of alkalinization of sample and homogenization with fine grain sand and anhydrous sodium sulfate, extraction with ethyl acetate in a glass column, transference of tyramine to hydrochloric acid, formation of the fluorescent complex with α-nitroso-β-naphthol and spectrofluorometric reading at 450/540 nm.

Total sulfur dioxide (method of Paul) and *volatile acidity* were determined following the 'Office International de la Vigne et du Vin' methods (OIV, 1969).

Malic and *lactic acids* were determined by enzymatic tests of Boehringer Mannheim (Cat. No. 139.068 and 139.084).

RESULTS AND DISCUSSION

Histamine and tyramine contents have been determined in 226 and 186 Spanish wines, respectively. The distribution of contents of both amines in these samples is shown in Fig. 1. It can be seen that over 50% of samples had histamine and tyramine levels under 2 mg/liter. Levels higher than 8 mg/liter were detected only for histamine. The range of histamine concentrations was from traces to 34·25 mg/liter. For tyramine the range was from traces to 7·80 mg/liter.

Most authors claim that the contents of biogenic amines is higher in red wines than in white ones (Marquardt & Werringloer, 1965; Saint-Blanquat & Derache, 1968; Lafón-Lafourcade, 1975; Tejedor & Mariné, 1979; Buteau *et al.*, 1984). This observation applies more to histamine than to tyramine. Figure 2 shows the distribution of histamine and tyramine in red, rosé and white wines. Concerning histamine, 86% of white wines and 91% of rosé

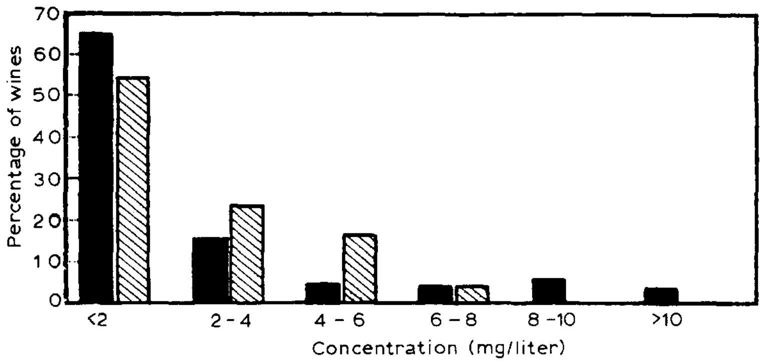

Fig. 1. Distribution of histamine ■ and tyramine ▧ contents in Spanish wines.

wines showed levels lower than 2 mg/liter. Levels higher than 4 mg/liter were found only in red wines. For tyramine, it can also be seen that most white and rosé wines had levels lower than 2 mg/liter (86% and 73%, respectively).

The average contents of histamine were 0·81 mg/liter in white wines ($n = 65$), 0·86 mg/liter in rosé wines ($n = 34$) and 4·07 mg/liter in red wines ($n = 127$). For tyramine, the average contents were 1·49 mg/liter in white

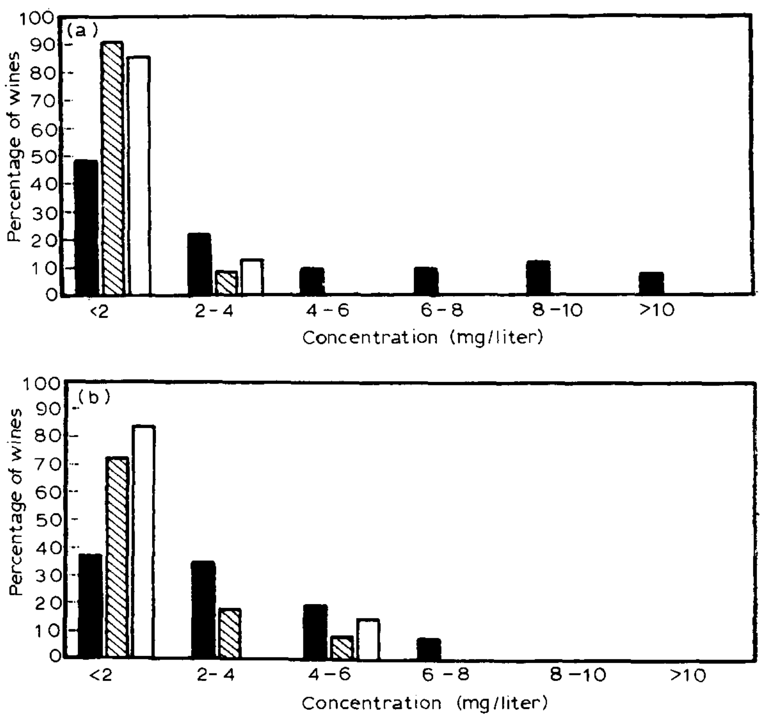

Fig. 2. Distribution of (a) histamine and (b) tyramine contents in red ■, white □ and rosé ▧ wines.

wines ($n = 52$), 1·66 mg/liter in rosé wines ($n = 23$) and 3·03 mg/liter in red wines ($n = 111$). By analysis of variance ($\alpha = 0{\cdot}05$) it has been proved that contents of both amines in red wines are higher than in white and rosé wines (histamine: $F_{tab} = 3{\cdot}00$, $F_{exp} = 24{\cdot}82$, DF = 2, 223; tyramine: $F_{tab} = 3{\cdot}00$, $F_{exp} = 17{\cdot}42$, DF = 2, 183). Between white and rosé wines there were not any statistically significant differences in contents of either amine (histamine: $F_{tab} = 3{\cdot}95$, $F_{exp} = 0{\cdot}08$, DF = 1, 97; tyramine: $F_{tab} = 3{\cdot}98$, $F_{exp} = 0{\cdot}24$, DF = 1, 73).

Since the formation of amines has been related to various factors linked with the manufacturing conditions of wine, an initial study was carried out in order to know if any kind of relationship could be established between biogenic amine content and total sulfur dioxide level, volatile acidity and MLF intensity. To this end, we studied red wines separately from white and rosé wines. For each of those two groups of wines, we established several subgroups according to the total sulfur dioxide levels, the volatile acidities, and the lactic acid/malic acid (L/M) and the lactic acid/potential lactic acid (L/Lp) ratios. These two ratios are quantitative expressions of MLF intensity that we propose in agreement with the criterion of Diez de Bethencourt (1972). L/Lp ratio will be lactic acid/(malic acid $\times$ 0·67 + lactic acid) (0·67 is the transformation factor from malic acid to lactic acid).

Range values for each parameter in the established subgroups of wines are not considered in legal regulations, but they correspond to parameter values generally considered high, medium or low. MLF intensity was evaluated using different ranges of values for red wines and for white and rosé wines, due to the different magnitudes of this second fermentation in the wines.

Tables 1 and 2 show the average contents of histamine and tyramine in white and rosé wines, and in red wines, respectively. Range values for each parameter corresponding to both groups of wines are also included. Figure 3 shows graphic representation of each amine's average content in each one of the established subgroups of wines (I, II and III), according to their total (a) sulfur dioxide level, (b) volatile acidity, (c) L/M ratio and (d) L/Lp ratio. Thus some possible relationships between amine levels and those parameters can be observed. To test if there were statistically significant differences in histamine and tyramine contents between the established subgroups of wines, analyses of variance ($\alpha = 0{\cdot}05$) were performed. Results of these analyses are shown in Table 3, and they are summarized as follows.

(a) In white and rosé wines, histamine and tyramine contents were not statistically different between the three subgroups of wines established according to their total sulfur dioxide level. On the contrary, in red wines, there were differences between the established three subgroups. Histamine and tyramine levels were higher in red wines with lower total sulfur dioxide level (subgroup I). Between subgroups II and III there were no differences.

TABLE 1
Histamine and Tyramine Average Contents in Subgroups of White and Rosé Wines, established according to their Total Sulfur Dioxide Level, Volatile Acidity and MLF Intensity

Subgroups of wines	*Histamine (mg/liter)*	*Tyramine (mg/liter)*
Total sulfur dioxide (mg/liter)		
I: <50	0·92 ($n = 18$)	1·56 ($n = 18$)
II: 50–150	0·69 ($n = 41$)	1·52 ($n = 41$)
III: >150	0·74 ($n = 12$)	1·84 ($n = 12$)
Volatile acidity[a] (g/liter)		
I: <0·50	0·42 ($n = 30$)	0·95 ($n = 30$)
II: 0·50–0·70	0·58 ($n = 28$)	1·42 ($n = 28$)
III: >0·70	1·91 ($n = 13$)	3·41 ($n = 13$)
L/M ratio		
I: <0·50	0·50 ($n = 52$)	1·13 ($n = 51$)
II: 0·50–1·00	0·36 ($n = 5$)	1·83 ($n = 5$)
III: >1·00	1·45 ($n = 21$)	2·53 ($n = 21$)
L/Lp ratio		
I: <0·10	0·55 ($n = 20$)	1·08 ($n = 20$)
II: 0·10–0·50	0·47 ($n = 32$)	1·12 ($n = 32$)
III: >0·50	2·17 ($n = 22$)	2·42 ($n = 22$)

[a] Volatile acidity is expressed as acetic acid.

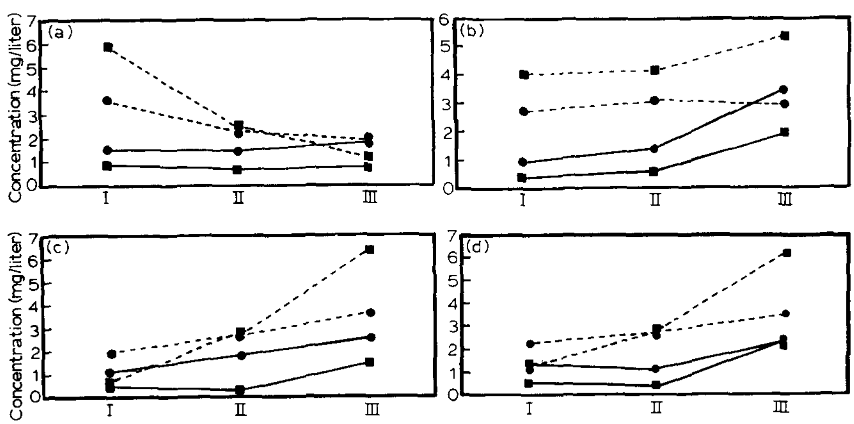

Fig. 3. Relationships between average amine contents and (a) total sulfur dioxide, (b) volatile acidity, (c) L/M ratio, and (d) L/Lp ratio. ■ Histamine, ● tyramine, —— white and rosé wines, - - - red wines.

TABLE 2
Histamine and Tyramine Average Contents in Subgroups of Red Wines, established according to their Total Sulfur Dioxide Level, Volatile Acidity and MLF Intensity

Subgroups of wines	*Histamine (mg/liter)*	*Tyramine (mg/liter)*
Total sulfur dioxide (mg/liter)		
I: <50	5·91 ($n=56$)	3·62 ($n=54$)
II: 50–150	2·54 ($n=31$)	2·30 ($n=30$)
III: >150	1·20 ($n=11$)	1·91 ($n=11$)
Volatile acidity[a] (g/liter)		
I: <0·50	4·01 ($n=11$)	2·70 ($n=11$)
II: 0·50–1·00	4·12 ($n=70$)	3·07 ($n=68$)
III: >1·00	5·29 ($n=17$)	2·94 ($n=16$)
L/M ratio		
I: <1·00	0·70 ($n=13$)	1·90 ($n=13$)
II: 1·00–10·00	2·72 ($n=39$)	2·66 ($n=38$)
III: >10·00	6·34 ($n=52$)	3·63 ($n=50$)
L/Lp ratio		
I: <0·70	1·06 ($n=17$)	2·14 ($n=17$)
II: 0·70–0·90	2·48 ($n=28$)	2·62 ($n=28$)
III: >0·90	6·20 ($n=57$)	3·50 ($n=57$)

[a] Volatile acidity is expressed as acetic acid.

TABLE 3
Results of the Comparison, by Analyses of Variance ($\alpha=0·05$), of Histamine and Tyramine Contents between Subgroups of Wines, established according to their Total Sulfur Dioxide, Volatile Acidity, and L/M and L/Lp Ratios

Analytical parameters	*Groups of wines*	*Histamine*			*Tyramine*		
		F_{tab}	F_{exp}	*DF*	F_{tab}	F_{exp}	*DF*
Total sulfur dioxide	White and rosé	3·14	0·37	2, 68	3·14	0·24	2, 68
	Red	3·10	8·70	2, 95	3·11	7·80	2, 92
Volatile acidity	White and rosé	3·14	18·59	2, 68	3·14	24·63	2, 68
	Red	3·10	0·42	2, 95	3·11	0·12	2, 92
L/M ratio	White and rosé	3·19	11·17	2, 75	3·20	10·19	2, 74
	Red	3·10	13·50	2, 101	3·10	6·29	2, 98
L/Lp ratio	White and rosé	3·20	4·91	2, 71	3·20	8·95	2, 71
	Red	3·10	13·14	2, 99	3·10	4·80	2, 99

This agrees with other authors' suggestions since high levels of SO_2 can act by inhibiting the growth of microorganisms with ability to generate amines.

(b) Concerning volatile acidity, we found that the subgroup of white and rosé wines with high volatile acidity (III) have also major amine contents. In red wines, that difference was not observed.

(c) Histamine and tyramine contents in wines also depend on MLF intensity. The established subgroups of wines according to their L/M and L/Lp ratios showed statistically significant differences in their histamine and tyramine contents. Those differences are present in both groups of wines (white and rosé wines, and red wines). Wines included in subgroups III, with highest values for L/M and L/Lp ratios, also showed highest histamine and tyramine contents.

Due to the existence of those statistically significant differences between subgroups of wines in their amine contents, analyses of correlation were made to check the signification of those relationships. Table 4 shows the results. For these correlation analyses the only wines considered were those for which all analytical parameters studied were determined. Only relationships with significance levels of 99·0% or more are considered. Of those analyses it can be concluded that:

(a) There is a very significant correlation (99·9%) between histamine and tyramine contents in all wines (white and rosé wines, and red wines).

TABLE 4

Linear Correlation Coefficient Values between Histamine and Tyramine Contents in Wines and between these and the Other Analytical Data (Sulfur Dioxide, Volatile Acidity, and L/M and L/Lp Ratios)

Variables	*Correlation coefficient values*	
	White and rosé wines (DF = 69)	*Red wines (DF = 93)*
Histamine–Tyramine	0·894 5**	0·752 0**
Histamine–Volatile acidity	0·531 3**	[a]
Tyramine–Volatile acidity	0·645 5**	[a]
Histamine–Total sulfur dioxide	[a]	(−)0·424 0**
Tyramine–Total sulfur dioxide	[a]	(−)0·433 0**
Histamine–L/M	[a]	0·729 4**
Tyramine–L/M	[a]	0·468 0**
Histamine–L/Lp	0·480 1**	0·521 8**
Tyramine–L/Lp	0·462 0**	0·339 0*

** $P = 99·9\%$.
* $P = 99·0\%$.
[a] Level of significance lower than 99·0%.

(b) In white and rosé wines there is a very significant correlation (99·9%) between histamine and tyramine contents and volatile acidity. In general, wines with high volatile acidity showed high amine contents. This correlation is not observed in red wines.

(c) Correlation (99·9%) between total sulfur dioxide level and biogenic amines has only been found in red wines. Highest amine contents were found in wines with low total sulfur dioxide level.

(d) Histamine and tyramine contents are correlated with MLF intensity in white and rosé wines, and in red wines. The best correlation (99·9%) was observed between the level of amines and L/Lp ratio. A very significant correlation (99·9%) between amine levels and L/M ratio was observed only in red wines.

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