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Biogenic Amines in Wines from Three Spanish Regions

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One hundred and sixty-three wines from La Rioja, Utiel-Requena, and Tarragona were analyzed to determine if there were any differences in the concentrations of six biogenic amines that are found in these three regions. The influence of grape variety, type of vinification, wine pH, malolactic fermentation, and storage in bottle on biogenic amine concentrations was studied. Results show important differences in putrescine and histamine concentrations among regions, varieties of grape, and type of wine; differences were less appreciable for the remaining biogenic amines studied. Low pH prevented biogenic amine formation. Malolactic fermentation and short storage periods in bottle (3−6 months) showed increases in histamine concentration, whereas longer periods of storage led to a general decrease in histamine. Several strains of lactic acid bacteria were isolated in this work, and their ability to form biogenic amines was assayed in synthetic media, grape must, and wine. Grape varieties, different types of winemaking, pH, and lactic acid bacteria may be responsible for the differences observed in the biogenic amine concentrations of the wines analyzed.

KEYWORDS: Biogenic amines; wine; malolactic fermentation; storage; grape variety; lactic acid bacteria; histamine; tyramine; phenylethylamine; putrescine

INTRODUCTION

Biogenic amines are organic bases endowed with biological activity that are frequently found in fermented foods and beverages. They are produced mainly as a consequence of the decarboxylation of amino acids. High concentrations of biogenic amines can cause undesirable physiological effects in sensitive humans, especially when alcohol and acetaldehyde are present (*1*, *2*). More specifically, histamine is known to cause headaches, low blood pressure, heart palpitations, edema, vomiting, and diarrhea (*1*, *3*). Tyramine and phenylethylamine can produce hypertension through the release of noradrenaline and norephedrine, respectively, which are vasoconstrictor substances (*4*). Putrescine and cadaverine, although not toxic themselves, aggravate the adverse effects of histamine, tyramine, and phenylethylamine, as they interfere with the enzymes that metabolize them (*5*, *6*).

Some amines, such as putrescine, may already be present in grapes (*7*), whereas others can be formed and accumulated during winemaking. Low potassium concentrations in soil have been reported to be responsible for high putrescine concentrations in plants (*8*, *9*). The main factors affecting its formation during vinification are free amino acid concentrations and the presence of microorganisms able to decarboxylate these amino acids. Amino acid concentration in grapes can be affected by fertilization treatments (*10*) and that in wines by winemaking treatments, such as time of maceration with skins, addition of nutrients, and racking protocols (*1*, *11*−*15*). The concentration of biogenic amines in wines depends on the presence and the concentration of yeast and lactic acid bacteria with decarboxylating activity (*11*, *16*) in addition to the precursors. The concentration of microorganisms is affected by physicochemical factors of wine such as pH, temperature, or SO_2 addition (*18*).

Many authors have implicated yeast and lactic acid bacteria as responsible for the formation of amines in wine (*12*, *14*, *15*, *18*, *19*). However, data are complex and contradictory, which suggest that more defined studies are necessary to elucidate which kind of microorganism is the major contributor. Several researchers have demonstrated that the amine content increases with microbial growth, specifically with that of bacteria, with biogenic amine content suggested as an index of quality or of poor manufacturing practices (*12*, *15*, *18*, *19*).

Biogenic amine content in wines may be regulated in the future following the newly implemented regulations by the U.S. Food and Drug Administration (FDA) for scombroid fish (*20*). Upper limits for histamine in wine have been recommended in Germany (2 mg/L), Belgium (5−6 mg/L), and France (8 mg/L) (*3*). Switzerland has established a limit of 10 mg/L as a tolerable value for histamine in wine (*21*).

To establish if there were differences in the biogenic amine contents of different Spanish wine-producing regions, Tempranillo wines from La Rioja, Tarragona, and Utiel-Requena were investigated. To discern if different grape varieties (Bobal, Garnacha, and Tempranillo) generate different amine concentrations, wines from three grape varieties of the same region (Utiel-Requena) were analyzed. To study the influence of the type of winemaking on biogenic amine concentration, white, rosé, and red wines from these regions were analyzed. In this work, we

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Table 1. Origins of Samples Analyzed in This Work

no. of wines analyzed	wine origin	grape variety	type of wine	time of sampling
35	La Rioja	Tempranillo	red	MLF[a] finished
2	La Rioja	Tempranillo	red	MLF completed by unknown commercial starter
5	La Rioja	Macabeo	white	MLF finished
8	La Rioja	Tempranillo	red	MLF in course
32	Utiel-Requena	Tempranillo	red	MLF finished
12	Utiel-Requena	Tempranillo	red	MLF in course
2	Utiel-Requena	Tempranillo	red	MLF completed by unknown commercial starter
16	Utiel-Requena	Bobal	red	MLF finished
2	Utiel-Requena	Bobal	red	MLF completed by unknown commercial starter
10	Utiel-Requena	Bobal	rosé	MLF in course
8	Utiel-Requena	Garnacha	red	MLF finished
8	Utiel-Requena	Macabeo	white	MLF in course
5	Utiel-Requena	Chardonnay	white	MLF in course
12	Tarragona	Tempranillo	red	MLF finished
6	Tarragona	Macabeo	white	MLF in course

[a] Malolactic fermentation.

also studied the influence of malolactic fermentation, storage in bottle, wine pH, and lactic acid bacteria.

MATERIALS AND METHODS

Origins of Wines Analyzed. The origins of the 163 samples analyzed are reported in **Table 1**. Samples originated from La Rioja, Utiel-Requena, and Tarragona. The wines were obtained from Tempranillo, Bobal, Garnacha, Macabeo, and Chardonnay grape varieties. The majority of wines were analyzed during or just after malolactic fermentation.

Evolution of Biogenic Amines after Alcoholic Fermentation and Storage. The evolution of biogenic amine content was investigated by sampling 12 young bottled wines from the Utiel-Requena region that originated from Bobal, Tempranillo, and Garnacha grapes. Samples were taken at different stages of vinification: before and after malolactic fermentation and at 3, 6, and 12 months during storage at a controlled temperature of 15 °C in the laboratory.

Isolation and Identification of Lactic Acid Bacteria. Lactic acid bacterium strains were isolated from wines reported in **Tables 4** and **5** by plating decimal dilutions on MRS (Scharlau Chemie S.A., Barcelona, Spain) plates supplemented with 0.5% L-cysteine and on MLO plates (*22*), which were incubated at 28 °C. Lactic acid bacteria were identified by means of fluorescence in situ hybridization (FISH) (*23*).

Determination of Biogenic Amines in Wines by HPLC. Histamine, tyramine, putrescine, cadaverine, phenylethylamine, and tryptamine were the six biogenic amines analyzed.

Samples of wine were centrifuged (13000 rpm or 10000*g*, 8 min), filtered through a membrane (regenerate cellulose, 0.45 μm pore size), and derivatized with orthophthaldehyde (OPA). Samples were injected into the HPLC system (Merck, Darmstadt, Germany) equipped with an L-Intelligent pump (Merck-Hitachi), an AS-2000A autosampler (Merck-Hitachi), a T-6300 column thermostat, and an L-7485 LaChrom fluorescence spectrophotometer (Merck-Hitachi). An excitation wavelength of 335 nm and an emission wavelength of 450 nm were used. OPA reagent was prepared as follows: 50 mg of OPA was dissolved in 2.25 mL of methanol; 0.25 mL of borate buffer 0.4 M (pH 10) and finally 0.05 mL of mercaptoethanol were added. A gradient of solvent A and solvent B was applied to a 100 RP-18 column (Merck-Hitachi) (25 cm $\times$ 5 μm) as follows: 0−20 min, 40% B isocratic, 1.1 mL/min; 20−45 min, 40−85% linear gradient, 1.1 mL/min. Solvent A consisted of 2.268 g of KH_2PO_4 and 14.968 g of $Na_2HPO_4 \cdot 12H_2O$ adjusted to pH 5.8 with H_3PO_4 and made up with deionized water to 1 L. Solvent B was 100% methanol.

Table 2. Average Concentrations and Standard Deviations of Biogenic Amines Found in Tempranillo Red Wines from La Rioja, Utiel-Requena, and Tarragona[a]

wine origin[a]	histamine (mg/L)	tyramine (mg/L)	putrescine (mg/L)	phenylethyl-amine (mg/L)
La Rioja (45)	8.2 ± 5.5	1.9 ± 1.1	47.3 ± 12.1	0.9 ± 0.4
Utiel-Requena (46)	2.4 ± 1.1	2.3 ± 0.8	7.5 ± 1.3	1.0 ± 0.4
Tarragona (12)	4.5 ± 3.1	1.8 ± 0.9	34.1 ± 10.5	0.8 ± 0.3

[a] The number of wines analyzed is given in parentheses. Wines with malolactic fermentation finished.

To evaluate the repeatability of the method, five independent analyses of biogenic amine concentrations in wine were performed, and the standard deviation was calculated.

The limit of detection (LD) of the method was calculated using the equation proposed by Miller and Miller (*24*). The LD is defined as the lowest concentration level of the element that an analyst can determine to be statistically different from an analytical blank. The repeatability standard deviation of measurements was 1.0%, and the LD was 0.1 mg/L.

Analyses of Biogenic Amines Produced by Lactic Acid Bacteria in Synthetic Medium, Wine, and Grape Must. The ability to produce different biogenic amines was analyzed in lactic acid bacteria isolated from wines (**Tables 4** and **5**). To do this, two synthetic media, wine and must, were used. Synthetic media are named biogenic amine production medium (BAPM) and modified histidine-decarboxylation medium (H-MDBmod). BAPM consists of the following (per liter): meat extract, 8 g; tryptone, 5 g; yeast extract, 4 g; glucose, 1.5 g; fructose, 1 g; Tween 80, 0.5 g; $MgSO_4$, 0.2 g; $FeSO_4$, 0.04 g; $MnSO_4$, 0.05 g; $CaCO_3$, 0.1 g; tyrosine, 2 g; histidine, 2 g; phenylalanine, 2 g; ornithine, 2 g; lysine, 2 g; tryptophan, 2 g; and pyridoxal phosphate, 0.25, pH 5.5. H-MDBmod contains the following (per liter): meat extract, 8 g; tryptone, 5 g; yeast extract, 4 g; glucose, 1.5 g; fructose, 1 g; Tween 80, 0.5 g; $MgSO_4$, 0.2 g; $FeSO_4$, 0.04 g; $MnSO_4$, 0.05 g; $CaCO_3$, 0.1 g; histidine, 20 g; and pyridoxal phosphate, 0.25 g, pH 5.2. Inoculation was performed by adding 0.1 mL of a mid-log phase MRS preculture medium into 10 mL of these media, which were left to grow at 28 °C until an OD_{600nm} of 0.5. This OD_{600nm} of 0.5 corresponds to 3.7×10^8 colony-forming units (cfu)/mL for *Lactobacillus hilgardii*, 4×10^8 cfu/mL for *Lactobacillus brevis*, 4.7×10^8 cfu/mL for *Pediococcus*, and 1.2×10^9 cfu/mL for *Oenococcus*.

BAPM contains a mixture of six amino acids, whereas H-MDBmod contains only histidine. When only the quantification of histamine is required, this last medium is preferred because the chromatographic analysis of this amine is enabled; the histamine peak partially overlaps with some of the other amino acids present in BAPM.

The confirmation of the ability to produce biogenic amines was performed in red wine (Bobal, Utiel-Requena, pH 3.5) and commercial grape must, supplemented with tyrosine, histidine, phenylalanine, ornithine, lysine, and tryptophan, 1 g/L each. These substrates were inoculated as described for synthetic media and incubated at 28 °C until the number of viable cells reached 10^7 cfu/mL.

To quantify the amines produced in the synthetic and natural media, samples were collected, centrifuged (13000 rpm or 10000*g*, 8 min), filtered through a membrane (regenerate cellulose, 0.45 μm pore size), and derivatized with OPA. The samples were then injected into the HPLC system as described above.

RESULTS

Quantification of Biogenic Amines in Tempranillo Wines from Different Wine-Producing Regions. The average biogenic amine concentrations of Tempranillo wines from Utiel-Requena, La Rioja and Tarragona are shown in **Table 2**. Putrescine was the most prominent biogenic amine in all of the

Table 3. Average Concentrations and Standard Deviations of Biogenic Amines in Wines from Three Grape Varieties with Malolactic Fermentation Finished Cultured in Utiel-Requena[a]

grape variety[a]	histamine (mg/L)	tyramine (mg/L)	putrescine (mg/L)	phenylethyl-amine (mg/L)
Tempranillo (46)	2.5 ± 1.2	2.6 ± 1.1	7.6 ± 2.1	1.2 ±0.5
Bobal (18)	2.3 ± 1.0	2.0 ± 0.8	3.5 ± 1.4	0.8 ± 0.3
Garnacha (8)	1.1 ± 0.3	0.8 ± 0.2	7.4 ± 2.1	0.6 ± 0.3

[a] The number of wines analyzed is given in parentheses.

analyzed wines. Histamine, tyramine, and phenylethylamine were also detected in decreasing concentration order. Important differences in putrescine concentration were observed among the three regions, with concentrations 7- and 5-fold higher in La Rioja and Tarragona, respectively, than in Utiel-Requena. A higher histamine concentration was also observed in La Rioja and Tarragona: 3 of 12 Tarragona wines and 16 of 45 La Rioja wines had a histamine concentration of >10 mg/L, but only 1 of 46 Utiel-Requena wines surpassed this concentration. However, the average tyramine and phenylethylamine contents were similar in all of the regions studied.

Low concentrations of cadaverine and tryptamine were found in the wines, always <0.5 mg/L. No differences were found for cadaverine and tryptamine concentrations among the wine-producing regions, grape varieties, and types of wine (red, rosé, and white).

Influence of Grape Variety on Biogenic Amine Concentration. The biogenic amine concentrations were quantified in Bobal (red and rosé), Garnacha (red), and Tempranillo (red) wines produced in Utiel-Requena. The average concentrations of histamine, tyramine, putrescine, and phenylethylamine are shown in **Table 3**. The histamine and tyramine concentrations were similar in the cases of Tempranillo and Bobal varieties and higher than in Garnacha. The putrescine concentration found in Bobal was slightly lower than those in Tempranillo and Garnacha. Phenylethylamine concentration was very similar in wines from the different grape varieties.

Influence of Type of Winemaking (Red, Rosé, and White) on Biogenic Amine Concentration. A total of 24 white, 10 rosé, and 129 red wines originating from the three different regions in the study were analyzed. In the white and rosé wines histamine and tyramine were <1 mg/L and phenylethylamine was <0.25 mg/L. No differences were observed in the concentrations of these three amines in wines from the different regions. However, the average putrescine concentrations varied in white wines from La Rioja (35 mg/L), Tarragona (29 mg/L), and Utiel-Requena (6.7 mg/L). Red wines showed histamine, tyramine, and phenylethylamine concentrations higher than those of white and rosé wines (see **Table 2**), but putrescine levels were similar in all three types of wines. Putrescine content seems to be more influenced by the geographical region and grape variety than by the type of winemaking.

The majority of white and rosé wines had not undergone malolactic fermentation, but in the cases of those that had, the histamine, tyramine, and phenylethylamine concentrations were close to those observed in red wines after malolactic fermentation. This information suggests that the lactic acid bacteria may be responsible for these higher values.

Influence of pH. The average pH values of the wines analyzed were 3.74 in La Rioja and 3.51 and 3.53 in Utiel-Requena and Tarragona wines, respectively. **Figure 1** shows that in wines that had undergone malolactic fermentation, higher

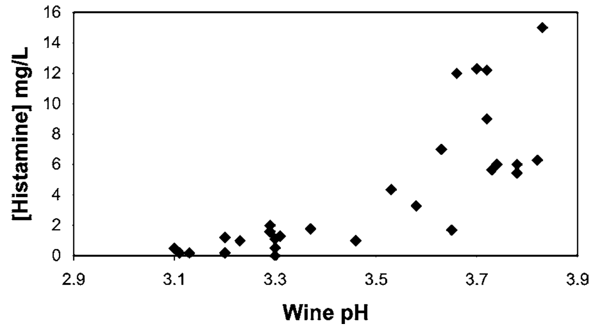

Figure 1. pH values versus histamine concentration from 28 different red, rosé, and white wines analyzed with malolactic fermentation accomplished.

histamine concentrations were observed at higher pH values. Wines with pH <3.6 showed histamine concentrations of <5 mg/L. This is consistent with the observation that white wines with the lowest pH values (average pH 3.11) exhibited the lowest histamine concentrations. However, the influence of pH on amine concentration is less evident for tyramine and phenylethylamine and was not observed for the rest of the amines (data not shown).

Influence of Malolactic Fermentation and Storage on Biogenic Amine Concentration. To establish more precisely the role of malolactic fermentation and storage on biogenic amine concentration, samples before and after malolactic fermentation and at 3, 6, and 12 months of storage were collected from 12 young wines from Utiel-Requena (**Table 4**). The evolution of the average concentrations of biogenic amines can be observed in **Figure 2**.

The biogenic amine showing a higher increase after malolactic fermentation was histamine; the others also increased, but to a lesser extent. During the first 6 months of storage in bottles, the histamine showed another increase, although smaller than that observed after malolactic fermentation. Consequently, a decrease in amine concentration was observed. Putrescine did not show any appreciable changes between the beginning and the end of the time period analyzed.

Quantification of Biogenic Amine Produced by Lactic Acid Bacteria. The ability to produce biogenic amines of lactic acid bacteria isolated from wines of the three different regions was analyzed in synthetic media, wine and must, and the results are reported in **Tables 4** and **5**. **Table 4** shows the results obtained with bacteria isolated from wines surveyed to establish a relationship between malolactic fermentation, storage, and biogenic amine content. The strains isolated were identified as *Oenococcus oeni*, *Pediococcus parvulus*, *Lactobacillus hilgardii*, and *Lactobacillus brevis*. Seven of the 12 wines analyzed contained histamine-producing lactic acid bacteria, with *O. oeni* strains being the most abundant. We found that *O. oeni* strains produced low histamine concentrations, whereas *P. parvulus* and *L. hilgardii* strains produced high levels in synthetic media. Strains of *L. brevis* were able to produce tyramine and phenylethylamine, but not histamine, in BAPM medium. We did not observe lactic acid bacteria capable of producing cadaverine, tryptamine, or putrescine in our experimental conditions. Those strains that were able to produce biogenic amines in synthetic media also produced them in wine or must, although the quantities were lower (see **Table 4**). The low production of tyramine and phenylethylamine produced by *L. brevis* strains in wine was due to their inability to grow. As a consequence, they were assayed in commercial must with added amino acids; the quantities of amines were recorded after

Table 4. Biogenic Amine Production in Synthetic Medium and Wine by Lactic Acid Bacteria Isolated from 12 Utiel-Requena Wines in Which the Effects of Malolactic Fermentation and Storage Were Studied[a]

			histamine (mg/L)		tyramine (mg/L)		phenylethylamine (mg/L)	
wine	grape variety	strain isolated	BAPM	wine	BAPM	wine	BAPM	wine
1	Bobal	*O. oeni* UR37	21	2.2				
2	Bobal	ND[b]						
3	Bobal	*O. oeni* UR181	12	5				
		Lb. brevis UR32			289	3	115	1.2
4	Bobal	*O. oeni* UR29	17	0.5				
		P. parvulus UR89	223	48				
5	Tempranillo	*O. oeni* UR54	22	6				
6	Bobal	*O. oeni* UR110	12	1.5				
		Lb, brevis UR16A			321	6.2	141	3.8
7	Bobal	*O. oeni* UR97	34	8				
8	Tempranillo	*Lb. hilgardii* UR241						
		P. parvulus UR86						
9	Tempranillo	ND						
10	Bobal	*O. oeni* UR102						
		P. parvulus UR96						
		Lb. hilgardii UR28	184	41				
11	Garnacha	*O. oeni* UR53B						
12	Garnacha	*P. parvulus* UR22						

[a] No strain was able to produce putrescine, cadaverine, and tryptamine. [b] ND, lactic acid bacteria were not detected.

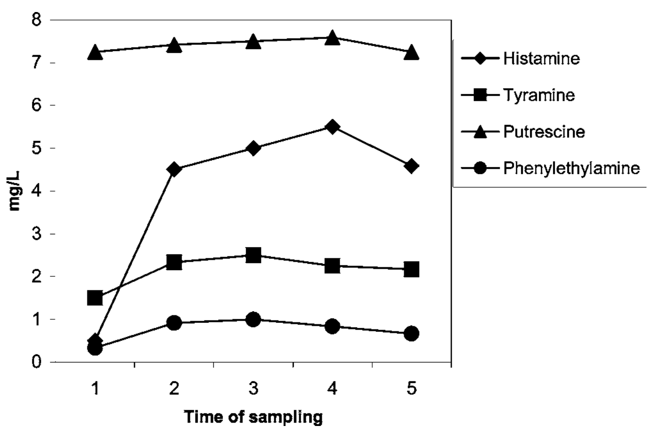

Figure 2. Evolution of histamine, tyramine, phenylethylamine, and putrescine concentration of wines from Utiel-Requena, before (1) and after (2) malolactic fermentation and 3 (3), 6 (4), and 12 (5) months of storage. Values represent mean contents observed in the 12 wines reported in **Table 4**.

Table 5. Histamine Concentration and Histamine-Producing Strains Found in Wines of Different Regions[a]

wine origin	histamine concn in wine (mg/L)	lactic acid bacteria isolated	histamine (mg/L) H-MDBmod	histamine (mg/L) wine
Utiel-Requena	4	*O. oeni* UR83B	17	3
Utiel-Requena	3	*O. oeni* UR10	21	7
La Rioja	11	*O. oeni* 318C	18	6.5
La Rioja	9	*O. oeni* 217B	22	11
Tarragona	1.5	*O. oeni* T26A	14	4.5
Tarragona	9	*O. oeni* T41	11	4
Utiel-Requena	8	*P. parvulus* UR89	223	48
La Rioja	20	*P. parvulus* 339C	212	34
Tarragona	21	*P. parvulus* T4	234	28

[a] The ability to form histamine in synthetic medium (H-MDBmod) and wine by lactic acid bacteria is also displayed.

growth: strain *L. brevis* UR32 produced 35 mg/L tyramine and 7 mg/L phenylethylamine, whereas *L. brevis* UR16A produced 43 and 11.5 mg/L, respectively.

Table 5 presents both the histamine content of the different wines from the three studied regions and the isolated strains that showed the ability to form histamine in H-MDBmod and in wine. Although more bacteria were isolated from these wines, only those that showed histamine production are recorded in the table. In these wines the histamine-producing strains belong to the species *O. oeni* and *P. parvulus*. We observed that *O. oeni* strains, as already demonstrated in **Table 4**, produced lower histamine in both synthetic medium and wine than *P. parvulus* strains. We did not find differences in the isolates' ability to produce histamine related to their geographical origin.

DISCUSSION

Putrescine was the most prominent biogenic amine found in wine; the other amines were present in substantially lower concentrations. Histamine and tyramine were the second and third most abundant biogenic amines. Tryptamine and cadaverine were always <1 mg/L. The results of this study are in agreement with other findings (*25*−*27*). In general, the biogenic amine concentration in Spanish wines is higher than in Portuguese wine (*28*) and similar to that in Bourgogne and Bordeaux wines (*27*).

Tempranillo wines from La Rioja and Tarragona showed the highest levels of putrescine and histamine concentrations. No differences were observed in tyramine and phenylethylamine contents. Differences in putrescine in musts can be explained, in part, by the chemical composition of soil, especially the potassium level, as has been demonstrated in grapevine leaves and eucalyptus (*8*, *9*). In addition, this amine could be formed from ornithine and arginine (via agmatine) by lactic acid bacteria. A higher content of these amino acids besides histidine could explain the higher level of putrescine and histamine in La Rioja and Tarragona wines. Also, it has been reported that wines with higher pH generally have higher amine concentrations (*25*). We observed that La Rioja wines exhibiting pH values >3.6 set a threshold for high histamine content (see **Figure 1**). This relationship between pH and amines is explained by the fact that at higher pH a greater number of bacteria can develop, thus increasing the probability of having strains able to form amines (*29*).

Some amines are normal constituents of grapes, being in variable amounts in different varieties. Among biogenic amines detected in grapes, putrescine and spermidine are usually abundant (20 and 45 mg/kg of fresh fruit, respectively), whereas ethanolamine, agmatine, cadaverine, spermidine, histamine, and tyramine have been found in lower amounts (*12*–*15*, *17*, *18*, *30*). High levels of putrescine, cadaverine, and spermidine have been reported in the pericarp of Cabernet-Sauvignon berries (*7*). In Utiel-Requena, Tempranillo wines showed the highest content of biogenic amines (average of 13.9 mg/L). Garnacha wines have 8.9 mg/L and Bobal, 6.6 mg/L, average (data deduced from **Table 3**). Putrescine is present in Tempranillo and Garnacha in 2-fold higher concentrations than in Bobal, whereas histamine and tyramine concentrations were very similar in Tempranillo and Bobal wines and lower in Garnacha. As the soils in which the grape varieties were cultured are similar in composition, they were all subjected to similar winemaking practices, and the bacteria able to form putrescine were not detected; differences in putrescine concentration could be attributed to the grape variety. Thus, Tempranillo and Garnacha musts probably had higher putrescine contents than Bobal; as we have not yet analyzed musts in this work, this is a hypothesis still to be confirmed. However, differences in histamine, tyramine, and phenylethylamine could be due to either different levels of precursors in the varieties or microbial formation, as can be concluded from the results recorded in **Table 4**.

Our results indicate that the type of vinification influences the amine content in wines; thus, white and rosé wines present lower amine concentrations than red wines. This may be attributed to various causes: lower amino acids content in berries because they are harvested earlier than red grapes, short or no maceration with skins, short contact time with lees, and no malolactic fermentation.

As derived from the results of this work, malolactic fermentation increases histamine concentration, which is probably due to the combination of two factors: the increase of amino acids in wine as a consequence of yeast lysis after alcoholic fermentation (*31*, *32*) and the proliferation of lactic acid bacteria with decarboxylase activity. In a medium poor in nutrients such as the wine, lactic acid bacteria can obtain energy to grow from decarboxylation of amino acid precursors (*33*). The fact that histamine was the amine which showed the highest increase after malolactic fermentation points to yeast lysis as the source of the precursor of histidine and to the presence of a high number of bacteria with histidine decarboxylase activity. Although *O. oeni* is the bacterium generally responsible for malolactic fermentation (*34*), other species such as *P. parvulus* and *L. hilgardii* can develop at the same time. *P. parvulus* and *L. hilgardii* strains that are able to form histidine exhibited a high histidine decarboxylase activity, but those of *O. oeni* showed a much lower activity in both synthetic media and wine (**Tables 4** and **5**). For this reason, a high number of *O. oeni* cells is required to explain the increase in histamine concentration when malolactic fermentation is performed by this species alone.

During storage the histamine concentration increased from 3 to 6 months. Gerbaux and Monamy (*35*) also found an increase in the concentration of histamine between 4 and 8 months after malolactic fermentation in Pinot Noir and Chardonnay wines and, then, a general decrease of concentrations was observed. The levels of histamine concentration, which decreased during storage, are probably due to degradation. In some fermented food it has been observed that the degradation of histamine and tyramine may be due to the action of amine oxidase present in foods, thus preventing their accumulation (*36*, *37*).

One way to prevent the problem of high biogenic amine concentration would be to reduce to a minimum the length of the processes that incorporate amino acids to must or wine as grape skin maceration and contact with lees, but this is impossible when aged wines are intended. Other factors such as grape variety or type of soil are not susceptible to change. For these reasons the control of this problem could be easily solved by inhibiting the growth of indigenous lactic acid bacteria and inoculating commercial selected *O. oeni* strains unable to produce biogenic amines. In support of this idea we have found lower concentrations of histamine, tyramine, and phenylethylamine in wines in which the malolactic fermentation was performed by a commercial starter.

A thorough study on amino acid decarboxylation by lactic acid bacteria under winemaking conditions would need to be done to estimate the real danger of lactic acid bacteria

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