

Biogenic amines in wines: Influence of oenological factors

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Abstract

Biogenic amines formation results from the decarboxylation of the corresponding amino acids by action of microorganisms. The presence of these compounds is considered by some authors a fundamental parameter for detriment of alcoholic beverages. The aim of this work was to assay the effect of some oenological factors (viticulture region, grape variety, anti-fungi treatment of grapes, fermentation activators, malolactic starters and storage on lees) from the point of view of their influence on the biogenic amines content of wines. According to our results, it was possible to show that the viticulture region affects the amounts of amines, since wines of some regions present higher contents of amines than wines from other regions. Grape varieties appear to influence the wine amines content. Commercial malolactic starters, after careful selection, should be added to the vinification process in order to decrease the formation of biogenic amines, since in our assays the wines that were inoculated with starters present lower amounts of biogenic amines. The wine storage on lees contributes for a biogenic amines increase.

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1. Introduction

Biogenic amines (BA) are naturally occurring compounds, ubiquitous in animals and plants. They are low-molecular-weight organic bases, aliphatic (putrescine, cadaverine, spermidine and spermine), heterocyclic (histamine and tryptamine), or aromatic (tyramine and phenylethylamine) (Lounvaud-Funel, 2001). These active compounds play important roles in normal mammalian physiology, like cell proliferation and differentiation (Bauza et al., 1995). BA are formed by decarboxylation of the corresponding amino acids by microorganisms through substrate-specific decarboxylase enzymes. This property is

not linked to a microbial species, usually it is strain dependent (Leitão, Teixeira, Barreto Crespo, & San Romão, 2000 and Moreno-Arribas, Polo, Jorganes, & Muñoz, 2003). This could, at least partially, explain why BA are randomly produced, in some wines they are detected sometimes presenting quite large values, while other ones show near trace values or do not present them at all. Decarboxylase enzymes are generally induced at acidic pH and therefore they have a possible role in maintaining pH homeostasis or enlarging the microbial growth period by detoxification of the extracellular medium (Marcobal, Rivas, Moreno-Arribas, & Muñoz, 2004 and Leitão et al., 2000).

The conditions that favour the occurrence of BA in wine dependent on time of must contact with grape skin, amino acid content at the initial and final phases of alcoholic fermentation and time of wine contact with yeast, (Vidal-Carou, Ambatlle-Espunyes, Ulla-Ulla, & Mariné-Font, 1990). The type and degree of ripeness of the grapes, the

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climate and soil of the viticulture area, and the vinification techniques also could contribute for the wine biogenic amines content (Ferreira & Pinho, 2006). BA 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 et al., 1990), as well as several volatile amines and polyamines (Feuillat, 1998).

Histamine, tyramine and putrescine are the BA found in higher concentration in wine, but cadaverine, phenylethylamine, isoamylamine can also be found (Bauza et al., 1995 and Silla Santos, 1996). Putrescine and cadaverine are normally associated with poor sanitary conditions of grapes (Leitão, Marques, & San Romão, 2005). Putrescine in grapevines has been also associated with potassium deficiencies in soil (Brodequis, Dumery, & Bouard, 1989). It is possible that this amine accumulates in the grapes, and consequently remains in the wine (Vidal-Carou et al., 1990 and Coton, Torlois, Bertrand, & Lonvaud-Funel, 1999).

The study of BA represents a concern for wine industry. From a toxicological point of view they can cause undesirable physiological effects in sensitive humans, especially if their metabolism is blocked or genetically altered (Ferreira & Pinho, 2006). They also can be a source of problems in commercial transactions since some countries have established maximum limits for histamine content in wine (Martín-Álvarez, Marcobal, Polo, & Moreno-Arribas, 2006).

The published studies concerning the toxicological effects in humans are contradictory. Some authors considered that the presence of BA in wine could be an important food safety problem due to some described implication of these compounds in cases of food intolerance and intoxication (Ferreira & Pinho, 2006; Martín-Álvarez et al., 2006; Marcobal, Martín-Álvarez, Polo, Muñoz, & Moreno-Arribas, 2006; Wantke, Gotz, & Jarisch, 1993, 1994, 1996). Under normal conditions, exogenous amines ingested as a part of the diet are absorbed and quickly transformed in the human organism by the action of the amine oxidases. However, when normal catabolic routes of amines are inhibited or a large amount of food containing BA is ingested, several physiological changes can occur, such as migraine headaches, nausea, hypo- or hypertension, cardiac palpitations, and anaphylactic shock (Silla Santos, 1996). According to other authors (Jansen, van Dusseldorp, Bottema, & Dubois, 2003; Kanny & Gerbaux, 2000; Kanny et al., 2001), no correlation was found between the occurrence of symptoms and the concentration of biogenic amines in wine samples, it appeared that red wine does not contain enough of key headache-producing compounds (histamine and tyramine) to be of significance in causing headaches and even psychosomatic reactions were admitted. Kanny et al. (1999) showed that the amount of histamine in wine has no clinical or biological effect in healthy subjects, and also emphasized the efficiency in man of the systems for degradation of histamine that is absorbed by the alimentary tract.

The wine industry is determined to reduce the presence of BA in wine. To better understand the prevention and control of the formation of these compounds, it is important to conduct critical analysis about the many factors associated with their development. The aim of this work was to study some oenological factors that could contribute to BA accumulation in wines.

2. Materials and methods

The oenological factors considered in this study were: viticulture region, grape cultivars, grape treated with different anti-fungi products, 2 fermentation activators (1 alcoholic fermentation activator and 1 malolactic fermentation activator), 2 commercial malolactic starters (CMS1 for red wines and CMS2 for white wines) and wine storage on lees. The wines used to study the influence of fermentation activators, commercial malolactic starters and storage on lees on BA concentration were produced on Estação Vitivinícola Nacional (EVN) during 2001 and 2002 harvest. The climatic conditions of 2001 and 2002 harvest were identical.

The wines were industrially elaborated in Portuguese wine-producing cellars. These wines were elaborated in stainless steel tanks following a typical red or white wine manufacturing process. The AF was carried out by indigenous yeast under controlled temperature.

At each sampling time, must and wine samples were collected and immediately frozen until analysis. Each assay was performed at least in duplicate and the mean values are reported.

2.1. Viticulture region

A total of 82 samples of red wines were produced in three different Portuguese regions (Douro, Dão and Alentejo) during 2003 and 2004 harvest. A total of 30 samples of red wines were elaborated in two wineries from Dão region on 2005 harvest.

2.2. Grape cultivars

The grape varieties used in this work were: Periquita, Espadeiro, Cabernet Sauvignon, Bastardo, Alfrocheiro and Tinta Miúda. The wines obtained from each cultivar were produced during the harvest of 1999.

2.3. Grape treated with different anti-fungi products

Only the wines from Periquita were used for this study during the 1999 harvest. In the vineyard, the cultivar Periquita was divided in 4 different groups, and each group was treated with a specific anti-fungi product. The fungicides tested were carbendazyme, iprodione and procymidone. The products were applied every 3 weeks in concentrations defined according to the climatic conditions observed. The 4th group had not received any anti-fungi treatment being considered the control group.

2.4. Fermentation activators

Two fermentation activators (nutritive factors) were tested: one activator of the alcoholic fermentation was added to the must and one activator of malolactic fermentation was added at the end of the AF. The fermentation activators (trade marks) were kindly supplied by the respective commercial supplier in Portugal.

2.5. Commercial malolactic starters

Two commercial malolactic starters were also tested (CMS1 in red wines; CMS2 in white wines) being added to the different wines at end of the AF, immediately after the first racking off. The malolactic starters (trade marks) were kindly supplied by the respective commercial supplier in Portugal. In these wines the MLF was conducted by the malolactic starters.

2.6. Storage on lees

At the end of MLF, the wines were divided in two parts. One part was stored on lees during 6 months in contact with the respective lees and the other one was stored for the same period without lees.

2.7. Biogenic amines analysis

Biogenic amines (histamine, tyramine, putrescine, cadaverine, phenylethylamine and isoamylamine) were analysed by reverse-phase high-pressure liquid chromatography (RP-HPLC) according to the method described by Vidal-Carou, Lazoh-Portolés, Bover-Cid, and Mariné-Font (2003). The RP-HPLC analysis was carried out with a fluorescence detector (excitation wavelength of 340 nm, and emission wavelength of 425 nm). The separations were performed on a Waters Nova-Pack C18 column. The derivatization process was post-column performed with *o*-phtaldialdehyde/2-mercaptoethanol (OPA/MCE) reagent. Samples were filtered (0.45 µm pore size filter; Millipore, USA) and then directly injected in duplicate onto the HPLC system. All the reagents used were HPLC grade.

3. Results

3.1. Viticulture region

Putrescine was the predominant amine in all of the analyzed wines (2004 harvest), at MFL end, from Douro, Dão and Alentejo viticulture regions. The wines from Douro and Alentejo presented the higher mean amounts of putrescine (10.9 ± 6.8 mg/L and 17.3 ± 5.0 mg/L, respectively), tyramine (2.8 ± 2.2 mg/L and 2.0 ± 1.8 mg/L, respectively) and histamine (5.0 ± 2.9 mg/L in both regions). The amounts of cadaverine, isoamylamine and phenylethylamine were always low. The wines from

Dão presented the lowest levels of BA and phenylethylamine was not detected in those wines (data not shown).

Wines from two wineries both in the same region (Dão) were studied, in order to determine the differences in BA. Wineries from the same region present different amounts of these organic compounds, especially, tyramine and putrescine. The amounts of cadaverine, isoamylamine and phenylethylamine never exceeded 1 mg/L. The wines produced in winery A present higher mean concentration values of tyramine (12.0 ± 2.3 mg/L) than wines from winery B (0.5 ± 0.1 mg/L of tyramine). The wines from winery B showed slightly higher amounts of putrescine than wines from winery A (5.0 ± 1.9 mg/L and 2.0 ± 0.9 mg/L, respectively). The amounts of histamine were identical in both wineries. Phenylethylamine was not detected in wines from winery B (data not shown).

3.2. Grape varieties

At the end of AF only small amounts of BA were detected in the several wines (data not shown). After MLF, in wines obtained from the 6 different grape varieties tyramine was the BA present in higher concentration. At the end of MLF, tyramine clearly increased especially in wines arising from the cultivars Alfrocheiro (31.5 ± 6.2 mg/L) and Espadeiro (24.4 ± 4.5 mg/L) (Fig. 1). The three wines obtained from Espadeiro, Bastardo and Alfrocheiro revealed low quantities of isoamylamine (between 2.5 ± 0.9 mg/L and 6.2 ± 2.1 mg/L). The wines arising from Piriquita, Cabernet Sauvignon, Bastardo and Tinta Miúda presented low levels of BA. The levels of cadaverine, histamine, phenylethylamine and putrescine were always low in all the wines.

3.3. Grape treated with different anti-fungi products

At the end of alcoholic fermentation no significant amounts of BA were detected in all the wines (data not shown). In this phase of the vinification process the levels of these compounds never exceeded 0.5 mg/L. After MLF achievement the control wines presented, on the whole, higher mean concentrations value of BA, than wines obtained from grapes treated with fungicides especially in the cases of isoamylamine (11.6 ± 0.6 mg/L), phenylethylamine (3.5 ± 0.4 mg/L) and tyramine (6.4 ± 5.0 mg/L), (Fig. 2). The wines from grapes treated with different fungicides present contents of tyramine between 1.7 ± 0.4 mg/L and 2.5 ± 0.3 mg/L, isoamylamine between 1.0 ± 1.2 mg/L and 4.1 ± 2.1 mg/L and the other BA never exceed 1.6 mg/L. The wines from grapes treated with carbendazyme presented the higher contents of BA and the wines from grapes treated with procymidone showed the lower levels of these compounds.

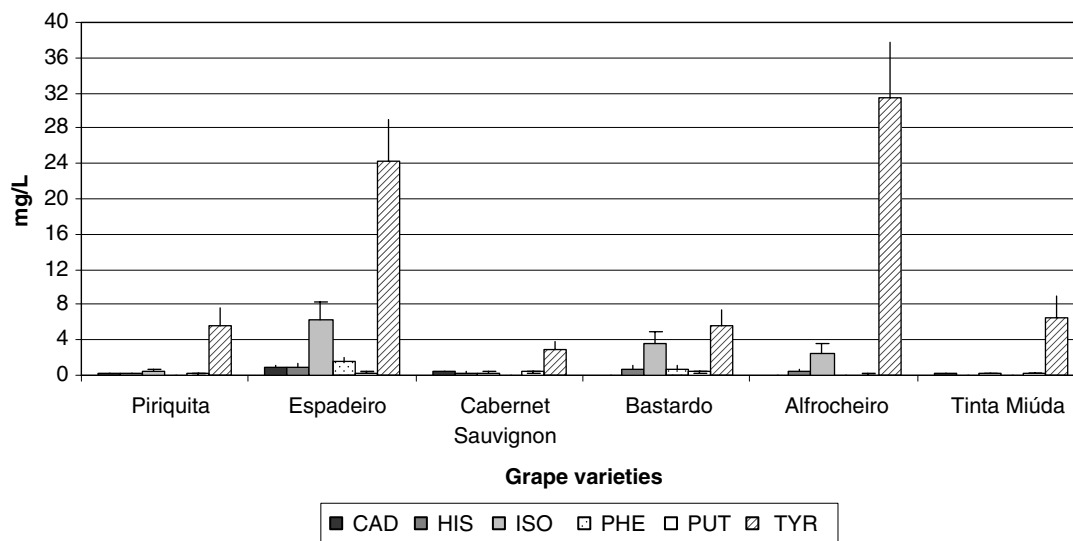


Fig. 1. Biogenic amines (mg/L) in wines produced from different grapes cultivars, at MLF end. Results are expressed as mean values \pm standard errors. ■ Cadaverine; ■ histamine; ■ isoamylamine; □ phenylethylamine; □ putrescine ▨ tyramine.

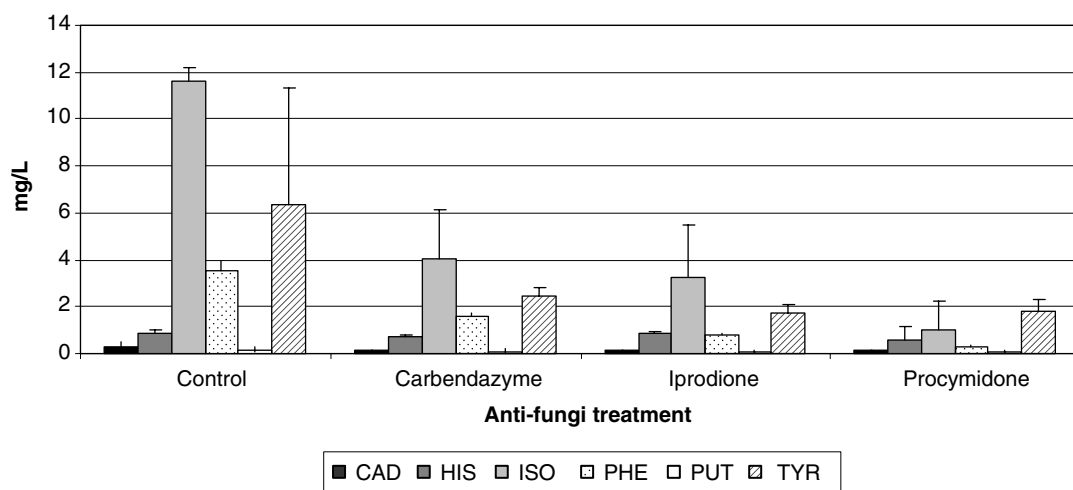


Fig. 2. Biogenic amines (mg/L) in wines from grapes treated with anti-fungi products, at MLF end. Results are expressed as mean values \pm standard errors. ■ Cadaverine; ■ histamine; ■ isoamylamine; □ phenylethylamine; □ putrescine; and ▨ tyramine.

3.4. Fermentation activators

The BA concentrations on wines added or not with the alcoholic fermentation activator are shown in Fig. 3a and those added or not with the malolactic fermentation activator are shown in Fig. 3b. Isoamylamine and tyramine were the BA present in higher concentration in must. Cadaverine, histamine, phenylethylamine and putrescine are present in concentration lower than 1 mg/L. At the end of AF a slight increase in tyramine and phenylethylamine, and a decrease in isoamylamine were observed. At the end of MLF, a slight formation of tyramine can be noticed. The amounts of cadaverine, histamine, phenylethylamine and putrescine were always very low.

The wines resulting from the addition of AF activator presented amounts of isoamylamine and tyramine slightly

higher than wines not added, especially at the end of MLF (Fig. 3a). The wines added with the MLF activator, presented similar amounts of BA than the control wines (Fig. 3b).

3.5. Commercial malolactic starters

The malolactic starter CMS1 was only tested in red wines according to the manufacturer instructions. Two months after MLF was finished, the inoculated wines presented lower concentration of BA than the control wines (Fig. 4a). Tyramine (18.9 ± 2.2 mg/L), putrescine (4.4 ± 2.4 mg/L) and histamine (4.3 ± 0.07 mg/L) were the BA present in higher concentration in control wines. For the inoculated wines only tyramine exceeds the 10 mg/L, while all the other ones presented very low levels.

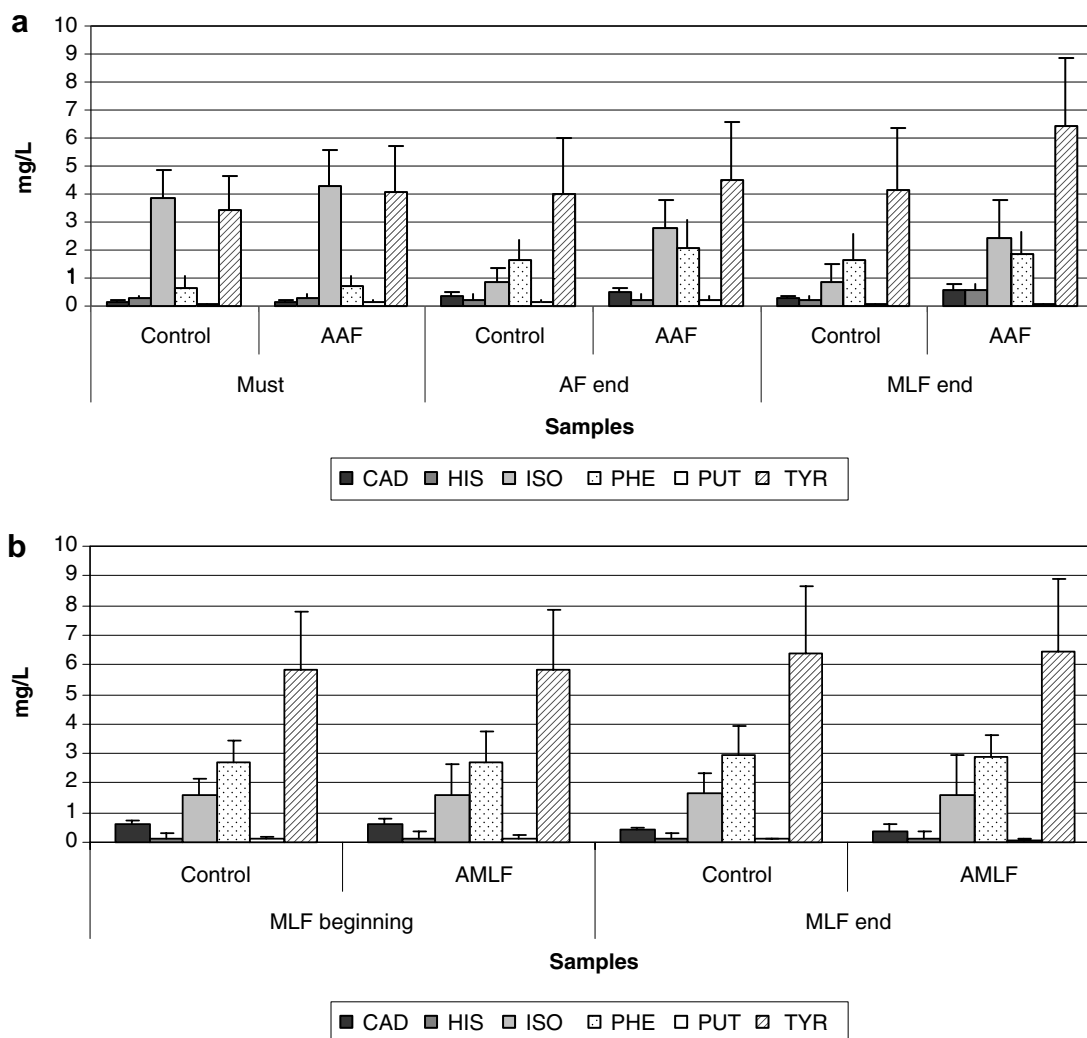


Fig. 3. Biogenic amines (mg/L) in wines added and not added with fermentation activators. (a) Wines added and not added with alcoholic fermentation activator. Sampling on Must, end AF and end MLF; and (b) wines added and not added with malolactic fermentation activator. Sampling on MLF beginning and MLF end. (AAF: wines added with AF activator; AMLF: wines added with MLF activator). Results are expressed as mean values \pm standard errors. ■ Cadaverine; ■ histamine; ■ isoamylamine; □ phenylethylamine; □ putrescine; ▨ tyramine.

The malolactic starter CMS2 was tested in white wines also according to the producer instructions. At the end of MLF the control wines presented higher mean concentrations value of tyramine (7.0 ± 2.2 mg/L) than the inoculated wines which presented 2.0 ± 2.0 mg/L (Fig. 4b). In this assay, two months after MLF was accomplished, the amount of tyramine and cadaverine in control wines increased while in inoculated wines only tyramine increased although the detected levels were always lower than in the control wines.

3.6. Storage on lees

As stated above, a portion of the red wine was stored for six months without lees and another portion was stored for the same period in presence of the respective lees. Two months after MLF the BA levels of histamine, isoamylamine, phenylethylamine and putrescine were identical in

both assays (Fig. 5). The tyramine and cadaverine levels were higher for wines stored on lees. Six months after MLF was possible to observe a slight increase of tyramine (from 6.3 ± 2.0 mg/L to 7.8 ± 2.4 mg/L) and a decrease of cadaverine in wines stored on lees. In the case of the control wines it was possible to observe that tyramine content increased markedly from ca. 0.2 to 5.5 mg/L after 6 months of storage, probably due to the presence of high producing-tyramine bacteria.

4. Discussion

Wine can be an ideal substrate for BA production, because its manufacturing process involves available free amino acids, the possible presence of decarboxylase-positive microorganisms, and some favorable environmental conditions that affect the growth of microorganisms and the activity of decarboxylase enzymes (Lounvaud-Funel,

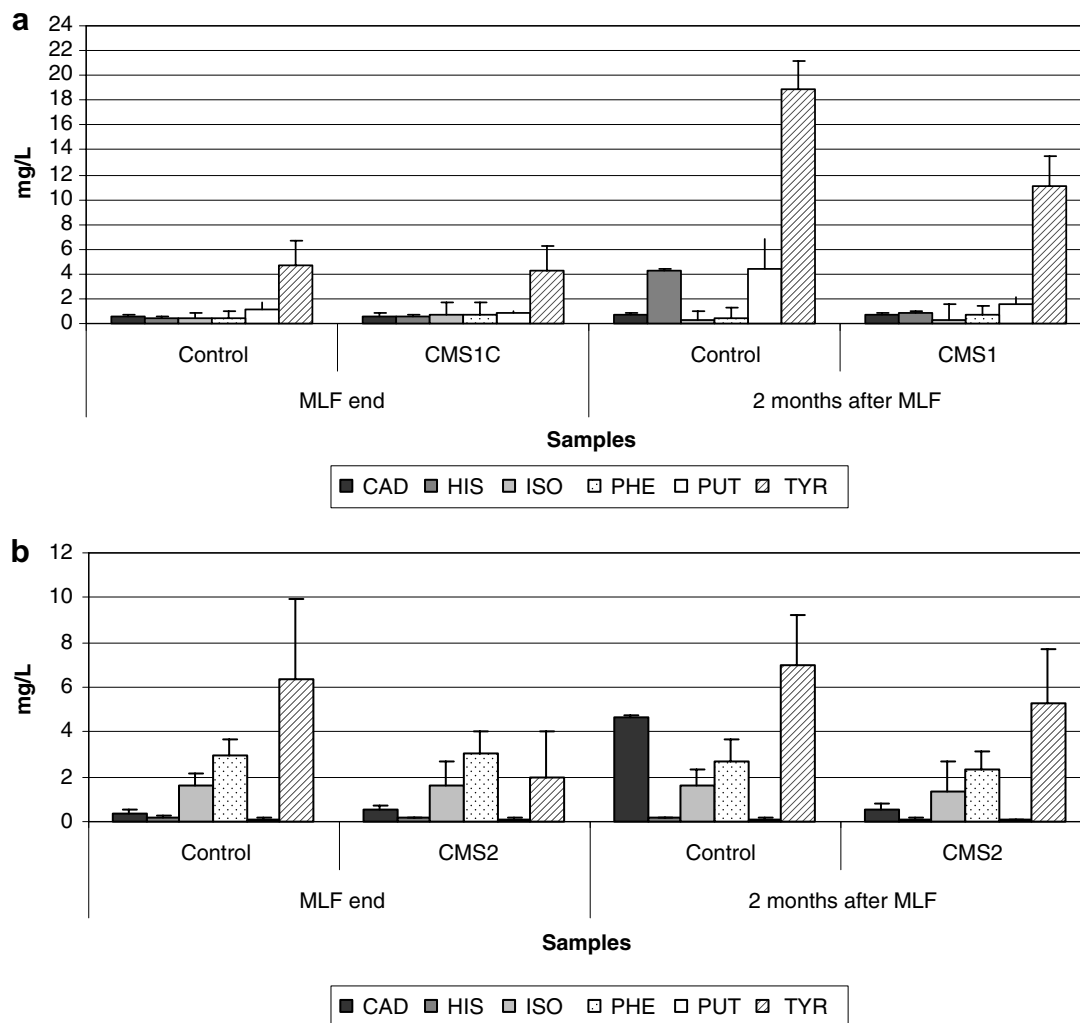


Fig. 4. Biogenic amines (mg/L) in wines added and not added with commercial malolactic starters. (a) Wines inoculated and not inoculated with commercial malolactic starter 1 (CMS1). Sampling: MLF end and 2 months after MLF; and (b) wines inoculated and not inoculated with commercial malolactic starter 2 (CMS2). Sampling: MLF end and 2 months after MLF. Results are expressed as mean values \pm standard errors. ■ Cadaverine ■ histamine; ■ isoamylamine; □ phenylethylamine; □ putrescine; and ▨ tyramine.

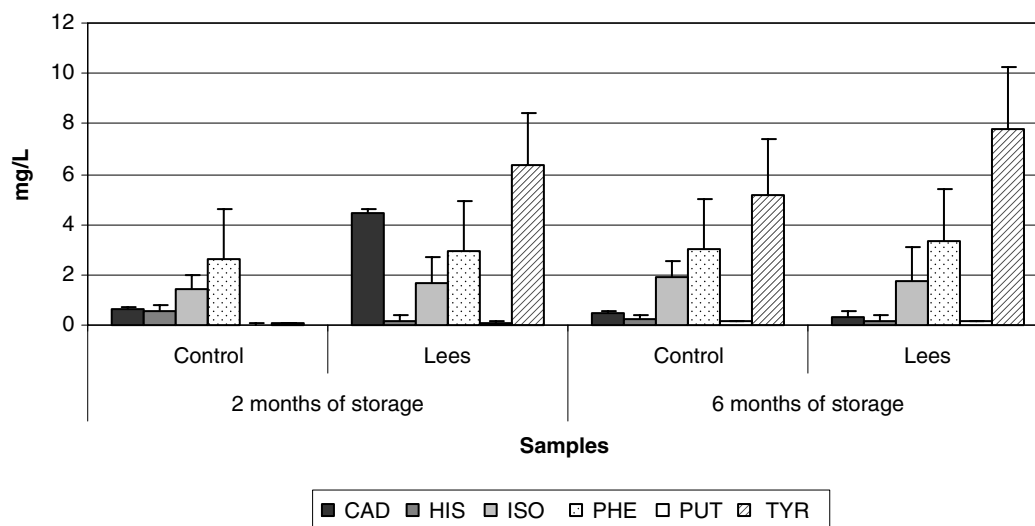


Fig. 5. Biogenic amines (mg/L) in wines storage and not storage on lees. Sampling: 2 months after MLF and 6 months after MLF. Results are expressed as mean values \pm standard errors. ■ Cadaverine; ■ histamine; ■ isoamylamine; □ phenylethylamine; □ putrescine; and ▨ tyramine.

2001). The definition of wine quality should have in attention its BA contents mainly due to commercial interests. The regulatory limits for BA in wines have not yet been established by OIV (“Organization Internationale de la Vigne et du Vin”), however some countries had create maximum limits for histamine content in wine (2 mg/L in Germany, 6 mg/L in Belgium, 10 mg/L in Switzerland and Austria, 8 mg/L in France and 4 mg/L in Holland) (Busto, Guasch, & Borrull, 1996). The maximum limit of biogenic amines generally considered to be safe for consumers is 10 mg/L (Loret, Deloyer, & Dandrifosse, 2005).

The major goal of this work was to study the effect of some oenological factors (viticulture region, grape variety, anti-fungi treatment of grapes, alcoholic fermentation activator, malolactic fermentation activator, commercial malolactic starter and storage on lees) on biogenic amines levels.

In the last few years it was possible to observe an increased of BA levels in Portuguese wines (Leitão et al., 2005). This can be attributed to climatic conditions, sanitary conditions of grapes, vinification method and or dominance of LAB species in the must.

Wines produced in two wineries from Dão region present different amounts of BA what is not surprising once the grapes origin, sanitary state and also winemaking process as well as indigenous microorganisms should be different.

Tyramine and isoamylamine were the BA present in higher amounts in wines obtained from 2003 harvest while in 2004 putrescine was the most important BA. The observed differences in BA in wines from different years can be due to the diversity of wine microorganisms that are naturally differently selected each year, probably due to climatic conditions and consequent viticulture/oenological practices. The observed difference in BA content between grape varieties is probably due to inherent types of amino acids composition and respective amounts in grape varieties. Other hypothesis to explain this difference is the natural bacteria microflora present on grapes.

The obtained results from anti-fungi treatment assays clearly show that fungal metabolic activity could have some influence in biogenic amine formation (especially isoamylamine) since, control wines made with grapes not treated with anti-fungi products present higher content of BA than wines obtained from treated grapes. To our knowledge, only a few studies reported information about the presence of amines in musts (Bertoldi, Larcher, & Nicolini, 2004 and Marcobal et al., 2006). The obtained results suggest that BA origin can be attributed either to the development of fungi in non treated grapes or to the activity of bacteria other than those normally present in healthy grapes.

In this study it was possible to detect isoamylamine and tyramine in the analyzed musts. Isoamylamine is normally associated to the activity of Enterobacteriaceae and fungi (Tavakkol & Drucker, 1983). At the end of AF the amounts of isoamylamine and tyramine showed a slight decrease when compared to the must contents, probably due to a co-precipitation with fine lees.

The addition of the AF and MLF activators to the wines does not appears to have any influence on BA formation, since those wines present almost the same amounts of BA as the control ones.

This study also confirmed that red wines present higher levels of BA than white wines, which can be explained by the different wine making techniques. On the contrary to white wines, red wines are prepared in presence of grape skins and grapes are submitted to some pressing. These practices tend to induce higher nutrients and microorganisms extraction in red musts. Therefore microbial activity is favoured and the obtained results are not surprising.

Previous works showed that some commercial malolactic bacteria did not produce BA (Moreno-Arribas et al., 2003). This work demonstrated that the application of commercial malolactic starters in wines was useful to reduce the BA amounts, since in the inoculated wines BA concentrations were significantly lower when compared with those not inoculated. In the case of the not inoculated wines the amounts of BA were higher, probably because MLF was conducted by indigenous malolactic bacteria higher BA producers. These results suggest that the use of well selected malolactic starters can minimise biogenic amines production.

Wine storage in presence of fine lees appears to contribute for the increase of BA in those wines. This was probably due to the contact of the wine with lees during which the proteins present in lees, mainly resulting from yeast autolysis, are hydrolysed to peptides and these peptides are later degraded further to amino acids and amines. These results are in agreement with those reported by Bauza et al. (1995).

From the overall results obtained along this study it is possible to conclude that the majority of the wines obtained appear to be safe either from a healthy or from a “legal” point of view as the levels of BA were normally low (<10 mg/L).

Additional investigation should be performed to understand and establish limits for biogenic amines levels in commercial wines. Control measures are needed to prevent BA formation keeping their levels in wine as low as possible what should obviously start by the choice of the lactic acid bacteria to be used as MLF starters. Also, further toxicological studies should be performed in order to analyze the potential impact of these compounds in human health.

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