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Amine concentrations in wine stored in bottles at different temperatures

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Abstract

Excessive concentrations of amines are undesirable in wine because they could be toxic or have a negative effect on aroma. Wine storage temperature has a decisive influence on product quality because a rise in temperature augments reactions within the wine. The aim of this study was to examine the evolution of biogenic and volatile amines during storage of wines in bottle at different temperatures. To do so, Chardonnay wine was stored in bottles at 4, 20 and 35 °C, during 105 days. The results showed that wine storage temperature had only a slight effect on the concentration of amines. The concentration of histamine stands out, as it was higher in the wine kept for 105 days at 20 °C than at the two more extreme temperatures of 4 and 35 °C. The formation or degradation of amines was mainly produced during the first 45 days of wine storage for all the temperatures under study.

Keywords: Amines; Wine; Bottle storage; Storage temperature

1. Introduction

Biogenic amines are undesirable in all food and drinks since, if they are absorbed in excessive concentration, they can induce respiratory distress, heart palpitation, hypertension, headaches and several allergenic disorders (Silla Santos, 1996). While individuals might have different sensitivities to the toxic actions of these substances, the toxic effects are usually related to the amount of food or drink ingested, to the total concentration of biogenic amines and to the consumption of ethanol and drugs (Sessa, Desiderio, & Perin, 1984). It has also been shown that simultaneous consumption of fermented foods and alcoholic beverages causes disorders, even when each single product could not be deemed hazardous. Volatile amines have little physiological significance but they can have a negative effect on wine aroma (Lehtonen, 1996).

The formation of biogenic amines is produced as a consequence of microbial decarboxylation of their respective free precursor amino acids (ten Brink, Damink, Joosten, & Huis in't Veld, 1990). Amino acid decarboxylation is an additional mechanism for energy generation for cells deprived from other substrates and it also constitutes part of the defence mechanisms against acid environments (Lonvaud-Funel, 2001; Lucas, Landete, Coton, Coton, & Lonvaud-Funel, 2003). The formation of volatile amines is different since it seems that they originate from amination of non-nitrogen compounds, such as aldehydes and ketones (Bauza, Blaise, Mestres, Daumas, & Cabanis, 1995).

Wine storage temperature has an influence on the quality of the product since the reactions which take place in bottled wine intensify with an increase in temperature (Boulton, Singleton, Bisson, & Kunkee, 1995). Although wine should never be kept at temperatures above 20 °C, there are occasions when the temperature will reach 30 °C or more for various reasons, such as climate or transport. Little work has been done on the relationship between temperature (both in fermentation and in storage) and the accumulation of amines in wine. Ough and Daudt (1981) found that medium fermentation temperature ranges result in a lower amine content in wine. Vidal-Carou, Codony-Salcedo, and Mariné (1991) did not find any increase in the concentration of histamine and tyramine

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during storage of wines under conditions of spoilage at various temperatures.

Given the importance of amines to both health security and wine quality and, as there are hardly any literature reports of the changes undergone by biogenic and volatile amines in wine during storage, the aim of this work was to study the evolution of amines during the conservation of wine in bottles at different temperatures. To do so, a white Chardonnay wine was kept at constant controlled temperatures of 4, 20 and 35 °C during 105 days and samples were periodically withdrawn to study the evolution of amines.

2. Materials and methods

2.1. Samples and vinification

The must used was *Vitis vinifera* var. chardonnay. The must was obtained by pressing at 0.5 atm. The must was treated with SO_2 (60 mg/l). Clarification was carried out by natural settling with the must in repose for 24 h at 10 °C. The sample was divided into two aliquots of 60 l and these were fermented in stainless steel tanks of 70 l capacity. Alcoholic fermentation were carried out at a controlled temperature of 17 °C, and lasted approximately 12 days (residual sugars <2.5 g/l). Malolactic fermentation took place by inoculating *Oenoccocus oeni* at a concentration of 1 g/hl (Uvaferm Alpha, Lallemand, Madrid, Spain). Wine from each stainless steel tank was treated by cold stabilization (−4 °C) and then filtered through a cellulose pad in a plate and frame filter; afterwards the wine was bottled in 750 ml bottles. Six bottles of wine (three from each stainless steel tanks) were kept in hot–cold incubator (Selecta, Barcelona, Spain) at constant controlled temperatures of 4, 20 and 35 °C for 105 days without light. To carry out the study, samples were taken from each bottle at different temperatures after 45, 75 and 105 days of wine storage. Analysis of amines from each sample was carried out in duplicate.

2.2. Preparation of sample and HPLC analysis of amines

To determine amines, the method described in detail by Torrea and Ancín (2001) was followed. Samples were cleaned by ultrafiltration with a Millipore Ultrafree MC cartridge. Next pre-column derivatization with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC) was carried out by adding 40 μl of a borate buffer solution to a 20 μl sample. Then 40 μl of AQC were added. Reagents were from AccQ-Fluor Reagent Kit (Waters, Milford, MA, USA). Analyses of the derivatized amines were performed with a Waters High-Pressure Liquid Chromatograph (Waters) equipped with two 510 pumps, a 717 Plus Autosampler, and a 474 Fluorescence Detector, using 250 and 395 nm as the excitation and emission wavelengths, respectively. Millenium 32 software was employed for chromatographic control. The amount of sample injected was 10 μl, and analyses were carried out in duplicate. A reverse-phase column (300 mm × 3.9 mm i.d.) was used, with a stationary phase of dimethyloctadecylsilyl bonded to amorphous silica. The column was set at 65 °C. The amines studied were histamine, tyramine, putrescine, cadaverine, spermine, dimethylamine, ethylamine, pyrrolidine, isopropylamine, isobutylamine, diethylamine, amylamine (not detected) and hexylamine. An individual stock standard solution of 2 g/l of each amine was prepared in HPLC-grade methanol (Scharlau, Barcelona, Spain) and stored in darkness at 4 °C. Solutions for further studies were prepared by diluting these stock standard solutions with water purified using a Milli-Q system (Millipore, Bedford, MA, USA). Standards were supplied by Aldrich (Gillingham, UK).

Precision of the method was calculated from the coefficient of variation (CV) and the results fluctuated between 1.8% and 9.7% for the different quantified amines. To calculate the CV, six derivatizations were carried out from one wine, and the concentrations of all amines were determined in each derivatized sample in duplicate. To examine the accuracy of the method, the recovery index was used. A known concentration of each amine was added to a previously analyzed wine sample and all the amines were quantified. This procedure was carried out for two different concentrations of the added amine standard. The recovered quantity was calculated from the difference between the measured concentration after adding the amines and the initial, endogenous concentration. The recovery index varied between 71% and 122% depending on the different amines analyzed.

2.3. Enological parameters and statistical analysis

Enological parameters are described by the Office International de la Vigne et du Vin (1990). The data were statistically analyzed using analysis of variance, with a confidence level of 95% and means were compared using the LSD analysis at the 0.05 probability level. The software used was SPSS v 11.5 (Chicago, IL, USA).

3. Results and discussion

3.1. Enological parameters

Table 1 shows the general parameters of the wine both before and after its storage for 105 days in bottles at 4, 20 and 35 °C. The table shows that the concentration of total SO_2 decreased by an appreciable degree in the wine kept at 35 °C unlike the wines which were stored at 4 °C and at 20 °C, when the concentration did not change. These results coincide with those of Ough (1985) who found that the loss of SO_2 increased when the storage temperature of the wine increased. Volatile acidity and pH were practically the same in all samples, independently of the wine storage temperature. Volatile acidity, although rather high, were found to be within the normal range for wine (Peynaud, 1993).

Table 1
Enological parameters of wine before and after storage during 105 days at different temperatures

	Wine	105 days of storage		
		4 °C	20 °C	35 °C
Free SO_2 (mg/l ± s)	12.0 ± 0.2	5.5 ± 0.4	6.3 ± 0.4	5.8 ± 0.4
Total SO_2 (mg/l ± s)	51 ± 7	49 ± 7	44 ± 7	31 ± 5
Volatile acidity (g/l[a] ± s)	0.7 ± 0.2	0.7 ± 0.1	0.8 ± 0.2	0.8 ± 0.1
Total acidity (g/l[b] ± s)	4.0 ± 0.1	3.9 ± 0.1	4.0 ± 0.1	3.9 ± 0.1
pH ± s	3.4 ± 0.1	3.4 ± 0.1	3.4 ± 0.1	3.6 ± 0.2
Alcohol (v/v %)	12.5 ± 0.1	12.2 ± 0.1	12.2 ± 0.1	12.1 ± 0.1

[a] Expressed as acetic acid.
[b] Expressed as tartaric acid.

3.2. Influence of temperature in the formation of biogenic amines

Fig. 1 shows that, in general terms, temperature had only a slight influence on the evolution of biogenic amines in Chardonnay wine during bottle storage. For the storage periods of 45 and 75 days, no significant differences ($p < 0.05$) were found in the concentrations of these amines, except in the concentration of spermine, among the samples stored at different temperatures. For the longer storage period (105 days) at room temperature, concentration of histamine in wine (2.2 mg/l) was significantly ($p < 0.05$) higher than that of wine stored at the more extreme temperatures of 4 and 35 °C (1.7 and 1.5 mg/l, respectively) (Fig. 1(a)). Putrescine, tyramine and cadaverine showed similar concentrations at different temperatures after a total of 105 storage days (Fig. 1(b)–(d)). The concentration of spermine diminished until it disappeared (Fig. 1(e)).

The concentration of histamine increased in a similar way (150%) for all the temperatures studied during the first 45 days of wine storage in bottle. Gerbaux and Monamy (2000) also found an increase of histamine in Chardonnay and in Pinot Noir wines when stored in bottles. This increase of histamine at the beginning of storage arises because, after the alcoholic and malolactic fermentations, microorganisms with decarboxylase activity remained in the product (Lonvaud-Funel, 2001). These microorganisms decarboxylate the amino acids freed at the end of fermentation, as a result of alteration of the plasmatic membrane in the yeasts in order to get energy (Kruger, Pickerell, & Axcell, 1992; Slaughter, Flint, & Kular, 1987). Coton, Torlois, Bertrand, and Lonvaud-Funel (1999) found that the activity of the histidine decarboxylase of *O. oeni* was not affected by the concentration of ethanol remaining in wine and was stable over time, even after the extinction of the viable bacteria cells. Our data would suggest that the activity of this enzyme is more favoured at room temperature than at extreme temperatures of 4 and 35 °C for the wine storage.

Putrescine was the most abundant amine in Chardonnay wine, as is the case in most red wines. Putrescine, tyramine

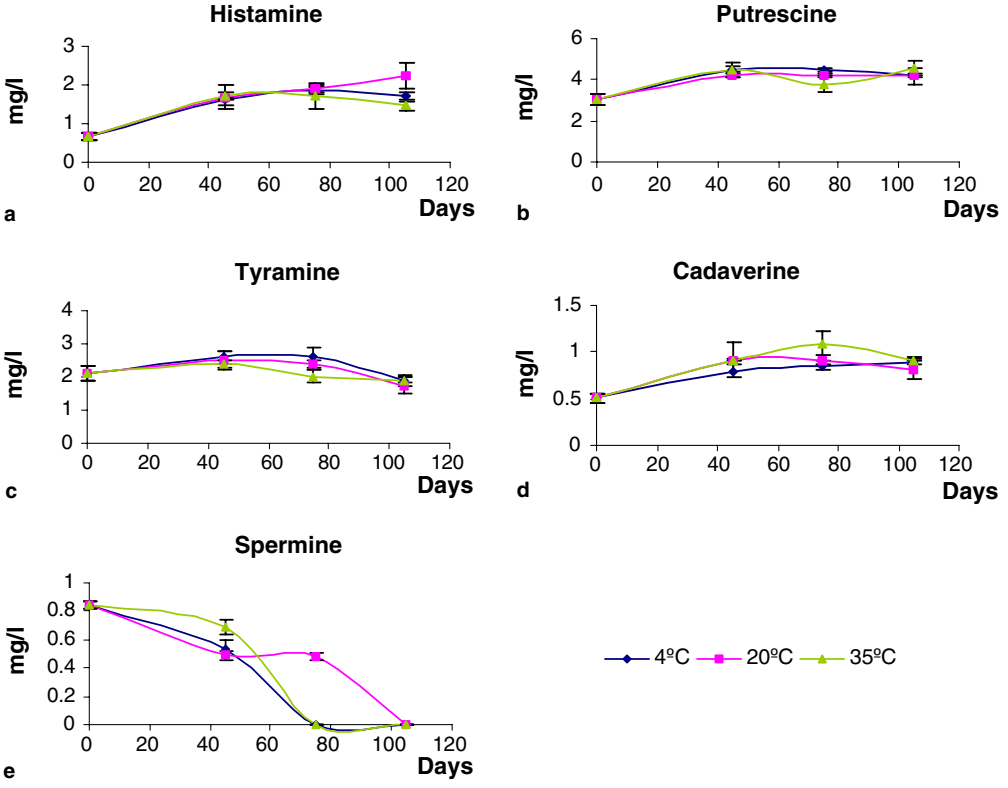

Fig. 1. Variations of biogenic amine concentration (mg/l) during bottled wine storage at different temperatures.

and cadaverine were formed during the first 45 days of bottled wine storage at different concentrations, but in a similar way at all temperatures under study (Fig. 1(b)–(d)). The formation of these amines, by residual microorganisms in wine, is related to decarboxylation of their precursor amino acids in order to obtain energy. From the results obtained, it seems that wine storage temperature did not have any effect on the activity of the enzymes which decarboxylate the precursor amino acids of these biogenic amines, which is just the opposite to what happens with histidine decarboxylase enzyme. Gerbaux and Monamy (2000) also observed an increase in the concentration of putrescine and tyramine in Chardonnay and Pinot Noir wines during storage in bottles. The concentration of tyramine diminished after 75 days of wine storage (Fig. 1(c)) due to the presence of tyramine oxidase in the wine. Some studies have found the presence of this enzyme in fermented beverages, including wine, and it also prevents the accumulation of tyramine in fermented foods (Enes Dapkevicius, Nout, Rombouts, Houben, & Wymenga, 2000; Leuschner, Heidel, & Hammes, 1998; Umezu, Shibata, & Umegaki, 1979; Voigt & Eitenmiller, 1978). It has been found that this enzyme has activity in wine although its greatest activity takes place at neutral or basic pH. Accordingly, Umezu et al. (1979) found oxidase activity below pH 4 in a synthetic medium. Putrescine and cadaverine maintain a stable concentration after 105 days of wine storage. These results agree with those of Jimenez Moreno, Torrea Goñi, and Ancín Azpilicueta (2003) who found that these amines were not degraded over the period of wine storage in barrels. Therefore, it would seem that there was no oxidation of these amines by oxidases in the wines.

The evolution of spermine (Fig. 1(e)) was completely different from the rest of the biogenic amines. This substance disappeared from the medium during wine storage at all the temperatures under study, although this occurred earlier at the extreme temperatures of 4 and 35 °C than at the room temperature of 20 °C. This would suggest that the formation of this amine by the residual wine microorganisms is not important for obtaining energy.

3.3. Influence of storage temperature in volatile amines

Fig. 2 shows the evolution of these compounds. These amines, except hexylamine, did not accumulate during wine storage. Just as occurred with biogenic amines, the main

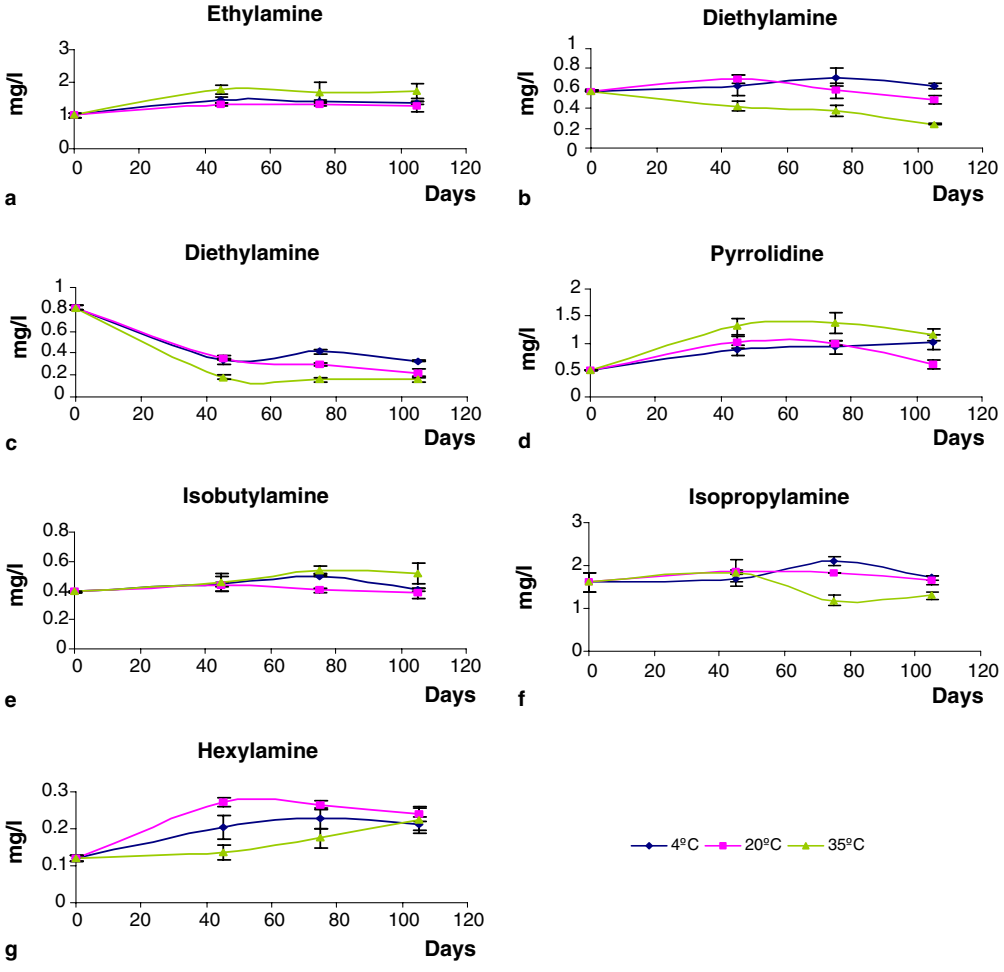

Fig. 2. Variations of volatile amine concentration (mg/l) during bottled wine storage at different temperatures.

modifications in these amines took place at the initiation of bottled wine storage. The concentration of ethylamine, pyrrolidine and hexylamine (Fig. 2(a), (d), and (g)) increased slightly at the start of the storage period, while the concentration of dimethylamine (Fig. 2(c)) diminished during the first 45 days of bottled wine storage and subsequently remained constant. The concentrations of isobutylamine and isopropylamine (Fig. 2(e) and (f)) showed no appreciable modifications during wine storage, while the concentration of diethylamine (Fig. 2(b)) diminished slightly in the wine stored at 35 °C. The increases in the concentration of ethylamine, pyrrolidine and hexylamine coincided with results found by Jimenez Moreno et al. (2003) for red wines aged in barrels. This increase could be due to the autolysis of the yeasts and/or a reductive amination of the corresponding aldehyde or the transamination of the aldehyde from an amino acid (Buteau, Duitschaever, & Ashton, 1984).

Ough and Daudt (1981) studied the evolution of volatile amines in storage of Pinot Noir and Riesling wines. These authors found that some amines were consumed while others were produced during aging, depending on the type of wine and fermentation temperature. In the present study, the wine storage temperature had little effect on the evolution of these substances. At the end of the wine storage period, the temperature had an effect on the concentration reached by these amines in a different way. The concentrations of diethylamine and dimethylamine were significantly higher ($p < 0.05$) in wine stored at 4 °C than in the samples stored at higher temperatures. However, the concentration of hexylamine was not affected by the temperature at the end of the storage period. Although there are few studies which associate the concentration of these amines with a negative effect on wine aroma, the high concentration of dimethylamine should be underlined, as it was always above 50 μg/l, the threshold limit for having a negative effect on beer aroma (Palamand, Hardwick, & Markl, 1969).

4. Conclusions

From the results obtained in this study, it may be concluded that wine storage temperature, in a reducing environment such as a bottle, has a slight effect on the evolution of amine concentration in this product. Only the concentration of histamine was higher in the wine stored at room temperature over 105 days, than in the wine stored at extreme temperatures of 4 and 35 °C. Moreover, the main changes in the concentrations of amines took place at the initiation of wine storage (the first 45 days) at all the temperatures studied.

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