EVOLUTION OF BIOGENIC AMINES CONTENT IN WINE DURING SAMPLE CONSERVATION – METHOD OPTIMISATION FOR ANALYSIS OF BIOGENIC AMINES•

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1. INTRODUCTION

The word “biogenic" means "created by life". The term "biogenic amines" is given for all the amines from the metabolism of living cells, animal, vegetable or microbial. Biogenic amines in wine are mainly of microbial origin (Lonvaud-Funel, 2001; Moreno-Arribas *et al*., 2003). These compounds may have an impact on human health (Ancin-Azpilicueta *et al*. 2008; Marques *et al*., 2008, Martin-Alvarez *et al*., 2006). Biogenic amines are naturally present in wine (mainly formed during malolactic fermentation by decarboxylation of amino acid precursors), and a regulation on the maximum level is currently under study. In addition, biogenic amines, besides their effect on human health, also play a role in masking flavors. In this context, it becomes essential to develop a reliable analytical method for rapid quantification of their presence in wines.

2. MATERIALS AND METHODS

The biogenic amines studied are primary amines, secondary or tertiary, aliphatic or aromatic. Many analytical methods are currently available (Önal, 2007). In our case, we focused on a method using the technology HPLC/DAD coupled to diethyl ethoxymethylenemalonate (DEEMM) derivatization following the method developed by Gomez-Alonso *et al*. (2007). Derivatization performance is calculated by adding an internal standard (2,4,6-trimethylphenethylamine hydrochloride). The technique has been validated with an alternative method of wine analysis for validation, quality control and assessment of uncertainties (OIV Oeno 10/2005). As an application of the proposed method, the content of biogenic amines in wines from the Rhône valley has been studied on 84 wines from three vintages (2005-2006-2007).

3. RESULTS AND DISCUSSION

As already described (Jimenez Moreno *et al*., 2003), the main biogenic amine is putrescine (tab. 1).

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Tab. 1 - Biogenic amines content (mg L^{-1}) in wines from the Rhone Valley (N = 84)

Amines	Mean	Standard Error	Max	Min
Histamine	5.08	3.07	14.05	<0.5
Methylamine	9.18	7.33	36.64	0.37
Ethylamine	3.84	1.63	10.46	1.66
Tyramine	4.70	2.98	12.35	<0.5
Purescine	13.27	8.93	48.72	3.71
Cadaverine	0.40	0.25	1.82	0.14
Phenethylamine	0.56	0.51	2.67	<0.5
Isoamylamine	0.28	0.24	1.23	<0.5

Regarding histamine, moderate amounts of 5 mg L^{-1} were found. All biogenic amines were found in small quantities, but it is worth noting the existence of wines including unusual quantities. No vintage effect could be demonstrated. According to the literature, preservation of samples does not appear to pose problem. During method validation, we nevertheless noticed changes, including lower concentrations in samples from the same wine analyzed several days apart (fig. 1). Based on this observation, it was therefore decided to study the samples conservation.

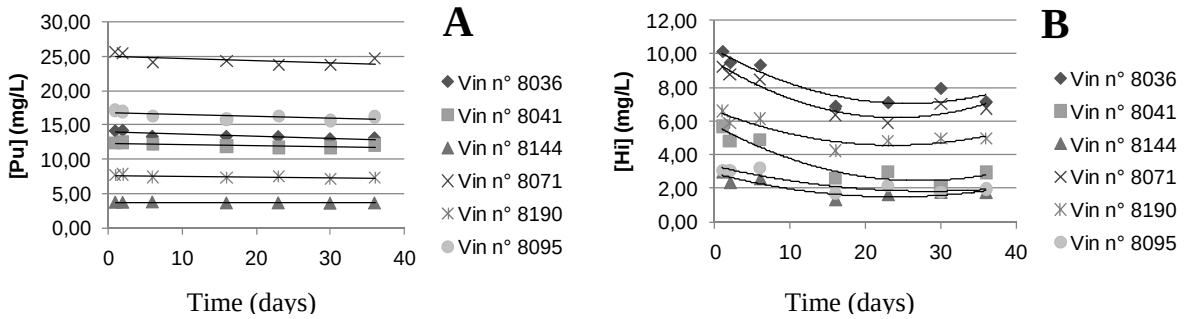

Fig. 1 - Evolution over time of the levels of putrescine (A) and histamine (B) samples before derivatization.

When observing the results, it quickly became clear that the concentration of histamine in different samples decreases substantially over time. However, in the remaining seven biogenic amines, their concentration remains substantially the same over time. Different storage temperatures of the sample were studied: no impact on this degradation has been established. Based on these observations, it appears that there is no effective way to preserve samples awaiting analysis.

It should be noted that instability in the time of histamine is a phenomenon already observed during aging in bottles (Jimenez Moreno *et al.*, 2003). The mechanisms involved in this decline are currently unknown. It therefore seemed interesting to study the evolution of the levels of biogenic amines in samples subjected to derivatization, to assess whether this method could be an attractive alternative (fig. 2). The results showed that the derivation allowed the samples stabilization.

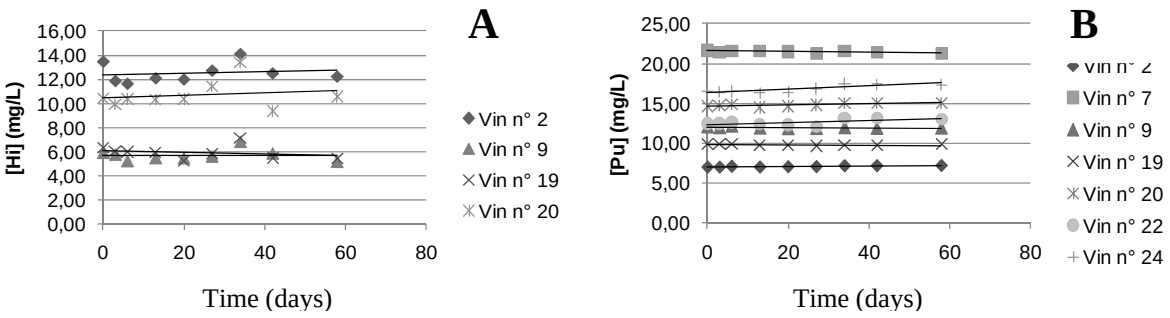

Fig. 2 - Evolution over time of the levels of histamine (A) and putrescine (B) samples after derivatization.

3. CONCLUSION

The method of analysis of biogenic amines by HPLC-DAD after Shunt DEEMM was chosen after comparison with existing methods (derivation pre or post column OPA) for the following reasons:

- limiting the costs of consumables: the column can allow the analysis of hundreds of samples, reagents are very stable and do not clog the column. It is therefore not necessary to group the analysis of samples;
- we have shown that histamine levels in wine may decrease by 30 % in the first month after receipt of samples. To counteract this problem, we studied the conservation of samples after derivatization. The results show that after two months of storage, changes of histamine is less than 10 % and stable for other biogenic amines of interest;
- this method allows the analysis of precursors of biogenic amines (amino acids) (Gomez-Alonso *et al*., 2007);
- biogenic amines derivatized by DEEMM were detected by a diode array detector at 280 nm. This equipment is generally more common in a laboratory, making the dissemination of the method easier.

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Abstract

The present paper reports the development of an optimized method for simultaneous analysis of 8 biogenic amines (Histamine, Methylamine, Ethylamine, Tyramine, Putrescine, Cadaverine, Phenethylamine, and Isoamylamine). It is based on a method developed by Gomez-Alonso *et al*. in 2007. The proposed analytical method has the following advantages: easy derivatization of wines, quantification of biogenic amines, and complete degradation of excess derivatization reagent during sample preparation to preserve column. It consists of reversed phase separation by HPLC and UV-vis detection of the aminoenones formed by the reaction of amino compounds with the derivatization reagent diethyl ethoxymethylenemalonate (DEEMM). The technique was confirmed

with an alternative oenological analysis method for the validation, quality control and uncertainty assessment (OIV Oeno 10/2005).

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