

EFFECTS OF SELECTED VITICULTURAL AND ENOLOGICAL FACTORS ON LEVELS OF 2-METHOXY-3-ISOBUTYLPYRAZINE IN WINES

EFFETS DE CERTAINS FACTEURS VITICOLES ET ENOLOGIQUES SUR LES TENEURS EN 2-MÉTHOXY-3-ISOBUTYLPYRAZINE

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Abstract : This paper examines the influence of viticultural and winemaking parameters in the 2-methoxy-3-isobutylpyrazine level in wines. Variety, state of maturity of grapes and maceration duration controlled decisively the amount of this compound.

Résumé : L'influence de certains facteurs viticoles et vinicoles sur les teneurs en 2-méthoxy-3-isobutylpyrazine dans quelques vins est examinée. Les analyses, effectuées par chromatographie en phase gazeuse et spectrométrie de masse, de vins du Bordelais de la récolte 1995, font ressortir, pour huit échantillons de vins issus du cépage Cabernet Sauvignon, une teneur moyenne deux fois plus élevée que pour six échantillons de vin provenant de la variété Merlot ; les teneurs moyennes sont respectivement de 10.6 et 5.2 ng/l. Par rapport aux vins du Bordelais, la teneur en 2-méthoxy-3-isobutylpyrazine pour deux vins grecs élaborés à partir du cépage Xynomavro de la région de Naoussa est légèrement plus élevée. Pour ce cépage, une récolte différée de deux semaines entraîne dans les vins une diminution d'environ 50 p. cent de la teneur en 2-méthoxy-3-isobutylpyrazine. Dans un essai, il a été obtenu une augmentation de la teneur en 2-méthoxy-3-isobutylpyrazine au cours de la macération. Également, par rapport aux vins de goutte, les vins de presse correspondants de Merlot et de Cabernet franc présentent des teneurs plus élevées en 2-méthoxy-3-isobutylpyrazine.

Key words : 2-methoxy-3-isobutylpyrazine, GC-MS, varieties, maturity, maceration

Mots-clés : 2-méthoxy-3-isobutylpyrazine, GC-MS, cépages, maturité, macération

INTRODUCTION

The 2-methoxy-3-alkyl-pyrazines are powerful flavorants that contribute to the characteristic aroma of many vegetables such as peas and bell peppers (MURRAY *et al.*, 1970 ; MURRAY and WHITFIELD, 1975).

2-methoxy-3-isobutylpyrazine was identified for the first time, in a Cabernet Sauvignon wine (BAYONOVE *et al.* 1975), and later tentatively in Sauvignon blanc wines (AUGUSTYN *et al.*, 1982). Therefore the herbaceous character of Sauvignon blanc and Cabernet Sauvignon wines was attributed

to the presence of 2-methoxy-3-alkyl-pyrazines (BAYONOVE *et al.*, 1975, LACEY *et al.*, 1991). Although these substances were present in the wines at very low contents, their threshold value is low (BUTTERY *et al.*, 1969). ALLEN *et al.* (1991) proposed for 2-methoxy-3-isobutylpyrazine a sensory detection threshold in water and white wine of 2 ng/l. Recently, KOTSERIDIS *et al.* (1998) found a detection threshold of 0.5 ng/l in water and of 10 ng/l in a red wine.

For many years, this compound was considered to be the impact compound of Cabernet Sauvignon and Sauvignon grapes. For the first time, ALLEN *et al.*

(1994) showed that this compound could be found in wines from the Saint-Emilion region made mainly from Merlot grapes. Later, HASHIZUME and UMEDA (1996) showed that Japanese Merlot wines contained approximately the same level of 2-methoxy-3-isobutylpyrazine, as Cabernet Sauvignon and Cabernet franc wines. KOTSERIDIS *et al.* (1998) showed that the levels of 2-methoxy-3-isobutylpyrazine in Merlot noir clones wines and Cabernet Sauvignon wines were in the same range. Furthermore, these authors showed that the levels of 2-methoxy-3-isobutylpyrazine in wines was influenced by the vintage. As reported by ALLEN *et al.* (1989), freshly fermented wines from Cabernet Sauvignon contained significantly higher levels of 2-methoxy-3-isobutylpyrazine than the corresponding grape juices prior to fermentation, probably due to the presence of ethanol during the maceration.

The aim of this paper was to study the influence of grape maturity and maceration on 2-methoxy-3-isobutylpyrazine levels in wines.

MATERIALS AND METHODS

I - WINE SAMPLES

1) Viticultural parameters

For the influence of cultivar assay, 14 wines from Merlot and Cabernet Sauvignon grapes, grown in 1995 vintage in various Bordeaux regions (Pomerol, Saint-Emilion, Graves, Moulis, Pauillac, Margaux, Saint-Estèphe), were analysed. The grapes came from vines with the same age approximately and the same cap management. The harvest was carried out at technological maturation.

The 4 Xynomavro wine samples (*Vitis vinifera* variety from Greece) were made with grapes of Xynomavro grown in 1995 in the region of Naousa in Northern Greece.

Totally 18 wines were analysed.

2) Winemaking parameters

The samples concerning the comparison of press and free-run-juice wines came from Saint-Emilion. After 13 days of maceration, the free-run juice wines were sampled and the press wines were produced by pressure of marc using a hydraulic press. One sample of Cabernet franc and one sample of Merlot sampled twice for analysis.

The final hot maceration trial was effected in two samples of Cabernet Sauvignon wines. After the fermentation procedure, the temperature of wine in two vinification tanks was set at 35°C for three days.

Concerning the influence of duration maceration trial, the experiment was settled in a winery of Graves. Samples of 50 l were sampled in the third, fifth and eighth day of fermentation-maceration. The wines were put into 50 l stainless steel tanks to finish their alcoholic fermentation. Fermentation temperature was kept about 25°C in all the tanks. After fermentation, the wines were placed in a carbonic atmosphere, before beginning malolactic fermentation. Malolactic fermentation was achieved with indigenous bacteria. When the latter was completed the wines were drained, 40 mg/l SO₂ were added, bottled and stored at 10°C.

Totally, 9 wine samples were analysed.

II - EXTRACTION OF VOLATILE COMPONENTS OF WINES

50 ml of the wine, previously spiked with 50 µl of 2-methoxy-3-methylpyrazine at 2.264 mg/l in anhydrous ethanol (final concentration in the analyzed sample of 2.264 µg/l) were analysed, according to the analytical method reported by KOTSERIDIS *et al.* (1998). The extraction of volatile compounds was carried out at room temperature using successively 4 ml, 2 ml and 2 ml of diethylether/hexane (1/1, v/v), with stirring (1 000 rpm) for 5 minutes by a magnetic stirrer. The organic layers were blended. 2 ml of this organic phase were concentrated 10 times under a nitrogen stream and the extract kept at -20 °C until analysed.

III - GAS CHROMATOGRAPHY - MASS SPECTROMETRY CONDITIONS

2 µl of the extracts were injected in a Hewlett Packard HP gas chromatograph 5890 series II equipped with a 5970 B mass selective detector and a 5990 A MS chemstation (HP-UX). The column used was a 50 m Carbowax 20M fused silica capillary column (Biochrom®) 0.25 mm i. d. and 0.2 µm film thickness. The splitless/split injection port was heated to 200°C. The split vent was opened after 30 sec. The carrier gas was Helium 55 Norme Aga®, and the pressure was 170 kPa with a linear velocity of 40 cm/sec at 40°C. The oven temperature was 60 °C (for 1 min), then ramped at 4 °C/min to 220°C and held for 30 min. All mass spectra were scanned in the SIM mode at 70 eV. The source and detector temperatures were set respectively at 250°C.

The ion of $m/z = 124$ was chosen for the quantitative determination of 2-methoxy-3-isobutylpyrazine and the ion of $m/z = 151$ was used as qualifier.

IV - VALIDATION OF THE METHOD

The R^2 coefficient of the standard curve was 0.996. The reproducibility was very satisfactory as a standard deviation of 0.8 ng/l for 7 extractions (injections in duplicate) of the same wine was found, which corresponded to a coefficient of variation of 5.3 p. cent (KOTSERIDIS *et al.*, 1998). The limit of detection was 2 ng/l, which was near to the sensory detection threshold of this compound (10 ng/l in red wines, KOTSERIDIS *et al.*, 1998).

RESULTS AND DISCUSSION

The analytical methods used up to date to estimate the concentration of the target compound either lacks sensibility and accuracy (HEYMANN *et al.*, 1986 ; BOISON and TOMLINSON, 1990), either has major drawbacks as the complicated isolation procedure demanding three steps of isolation and special apparatus (HASHIZUME and UMEDA, 1996 ; HARRIS *et al.*, 1987).

In this study, we used the analytical method reported by KOTSERIDIS *et al.* (1998) for the analysis of varietal volatile compounds, which is a convenient method of isolation (demanding less than 30 min. of isolation, and common liquid/liquid extraction procedure) and quantification by GC-MS in SIM mode, to study the different viticultural and winemaking factors influencing the amounts of the target compound in the wines.

I - VITICULTURAL PARAMETERS

The influence of cultivar, and grape maturity on the content of 2-methoxy-3-isobutylpyrazine in the corresponding wines was studied. 2-methoxy-3-isobutylpyrazine contents in wines, depended on grape cultivar as shown in table I. The levels were higher in Cabernet Sauvignon wines (mean value 10.6 ng/l), than in Merlot wines (mean value 5.2 ng/l), in spite of the same type of cap management of the vine. As showed in table I, the maximum level of Merlot wines corresponded to the minimum content of Cabernet Sauvignon wines. The values presented in table I are mean values of two analysis for each sample.

The influence of grape maturity on Xynomavro cultivar was studied by analysis of Xynomavro wines.

TABLE I
Contents (ng/l) of 2-methoxy-3-isobutylpyrazine of Merlot and Cabernet Sauvignon wines (n = 2)

Tableau I - Teneurs (ng/l) en 2-méthoxy-3-isobutylpyrazine dans quelques vins de la récolte 1995 issus des cépages Merlot et Cabernet Sauvignon (n = 2)

Variety	Region	2-methoxy-3-isobutylpyrazine (ng/l)
Merlot	1 Pomerol	3,8
	2 Saint-Emilion	3,7
	3 Graves	4,3
	4 Pomerol	4,5
	5 Moulis	7,1
	6 Pauillac	7,5
Mean of Merlot		5,2
Cabernet Sauvignon	1 Margaux	8,5
	2 Margaux	8,9
	3 Margaux	9,2
	4 Graves	10,2
	5 Saint-Estèphe	10,3
	6 Saint-Estèphe	10,8
	7 Moulis	13,2
	8 Pauillac	14
Mean of Cabernet Sauvignon		10,6

TABLE II
Influence of grape maturity on 2-methoxy-3-isobutylpyrazine levels (ng/l) of Xynomavro wines (n = 2)

Tableau II - Influence de la date de récolte des raisins du cépage Xynomavro sur les teneurs en 2-méthoxy-3-isobutylpyrazine des vins (n = 2)

Vineyard	Harvest date	2-methoxy-3-isobutylpyrazine (ng/l)
A	10 september	33
	25 september	15
B	11 september	31
	26 september	17

A 15 days delay of the harvesting decreased by half the levels of 2-methoxy-3-isobutylpyrazine (table II). The values presented in table II are mean values of two analyses for each sample.

II - WINEMAKING PARAMETERS

Usually press wines are characterised by an herbaceous character. The free run juices of a Merlot wine and a Cabernet franc wine were compared to their corresponding press wines. The contents in the press wines (mean of two samples for each cultivar) were always higher and furthermore exceeded the sensory detection threshold (table III). That was due to the

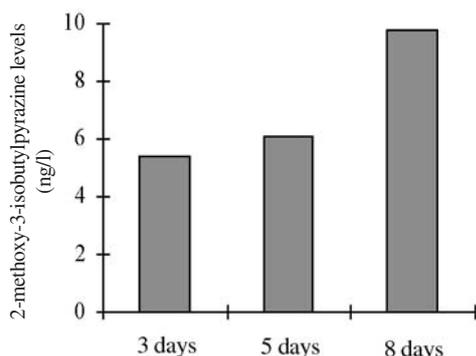


Fig. 1 - Evolution of 2-methoxy-3-isobutylpyrazine (ng/l) during maceration (n = 2)

Fig. 1 - Évolution des teneurs (ng/l) en 2-méthoxy-3-isobutylpyrazine pendant la macération (n = 2)

fact that almost half the quantity of 2-methoxy-3-isobutylpyrazine was located in skins of the berries (HASHIZUME and UMEDA, 1996) and thus could be extracted due to the partial destruction of the skins during the pressing process and the presence of ethanol during the maceration.

The final hot maceration was examined in two samples of Cabernet Sauvignon wines, but no difference was observed in 2-methoxy-3-isobutylpyrazine content with the control ones. That was probably due to the previous extraction of 2-methoxy-3-isobutylpyrazine during the fermentation and maceration by alcohol and the pumping over. Thus the maceration time was examined for Merlot wines. The wines were sampled 3, 5 and 8 days from the start of the maceration, during the alcoholic fermentation (mean of two samples for each date). The highest content of 2-methoxy-3-isobutylpyrazine was found in the third sample, as shown in figure 1.

CONCLUSION

The influence of some viticultural and winemaking parameters on the contents of 2-methoxy-3-isobutylpyrazine in some wines was studied. It was showed that the contents of 2-methoxy-3-isobutylpyrazine was influenced by variety and also that harvest date could influence the levels of this varietal substance in the corresponding wines of Xynomavro cultivar. As this substance was partly located in the skin or flesh of the berries, the pressing intensity, the frequency of pumping overs per day and the duration of maceration could influence the levels of this compound. Thus the analytical determination of this compound could be used to control grape maturity and skin contact time.

TABLE III

Comparison of 2-methoxy-3-isobutylpyrazine levels (ng/l) of Merlot and Cabernet franc free run juice and their corresponding press wines (n = 2)

Tableau III - Comparaison des teneurs (ng/l) en 2-méthoxy-3-isobutylpyrazine de vins de presse et de vin de goutte de Merlot et de Cabernet franc (n = 2)

Cultivar	Wine	2-methoxy-3-isobutylpyrazine (ng/l)
Merlot	Free-run-wine	5,1
	Press wine	10,8
Cabernet franc	Free-run-wine	8,5
	Press wine	12,1

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