

PYRAZINE CONTENTS IN FOUR RED GRAPE VARIETIES CULTIVATED IN A WARM CLIMATE

TENEURS EN PIRAZINE DE QUATRE CÉPAGES CULTIVÉS SOUS UN CLIMAT CHAUD

Raquel ROMERO, J.L. CHACÓN*,
E. GARCÍA and J. MARTÍNEZ

Instituto de la Vid y del Vino de Castilla-La Mancha (IVICAM),
Ctra. Toledo, Albacete s/n, 13700 Tomelloso, Espagne

Abstract : We analysed 2-methoxy-3-ethylpyrazine and 2-methoxy-3-isobutylpyrazine content in 4 grape varieties cultivated in Castilla-La Mancha. A sensory analysis of the wines obtained from these grapes was also performed. The results revealed the presence of the two methoxypyrazines studied in all four varieties, in concentrations lower than those published for these varieties in regions with cooler climates. Significant differences were observed in 2-methoxy-3-isobutylpyrazine content between the different varieties: Cabernet-Sauvignon>Merlot>Syrah>Cencibel. The correlation between 2-methoxy-3-isobutylpyrazine content and the « green pepper » descriptor suggests a perception threshold at 3 ng/L in young wines.

Résumé : Ce travail a permis d'analyser la teneur en 2-méthoxy-3-éthylpyrazine et 2-méthoxy-3-isobutylpyrazine dans quatre variétés de raisins cultivés à Castilla La Mancha au moment optimum de maturité : Cencibel, Cabernet Sauvignon, Merlot et Syrah. Le raisin a été cueilli à 7 emplacements différents dans la région de Castilla - La Mancha pour obtenir un ensemble de 23 échantillons. La teneur en 2-méthoxy-3-éthylpyrazine et 2-méthoxy-3-isobutylpyrazine a été déterminé en calculant la moyenne des deux échantillons les plus proches au moment de la maturité technologique dans chaque parcelle. Pour la préparation des échantillons à analyser, on pressé 1 000 grammes de raisin auxquels on a ajouté de la 2-méthoxy-3-méthylpyrazine comme étalon interne, jusqu'à obtenir une concentration finale de 18 ng/kg de raisin. Le moût obtenu a été soumis à une extraction liquide-liquide à l'aide d'un mélange de deux solvants (pentane et dichlorométhane) dans une proportion de 60:40 respectivement, et l'extrait résultant a été concentré par distillation en colonne Vigreux jusqu'à 2 mL. Les échantillons ont été analysés par GC/MS.

Pour l'analyse sensorielle des vins élaborés avec du raisin provenant des mêmes parcelles où ont été prélevés les échantillons, on a appliqué la méthode du Profil Sensoriel. Les échantillons ont été analysés par un groupe de dégustateurs préalablement entraînés, avec un critère exclusivement aromatique et non gustatif, en portant une attention particulière au descripteur « poivron vert ».

Finalement, avec les données obtenues des 23 échantillons, on a réalisé une analyse de variance et on a appliqué le test de Student-Newman-Keuls pour vérifier s'il existait des différences significatives entre variétés, ainsi qu'une étude de régression entre le contenu en pyrazine et l'intensité de l'arôme de poivron vert.

Les résultats ont révélé la présence, dans les quatre variétés, des deux méthoxypyrazines étudiées dans des concentrations inférieures à celles publiées dans des régions de climat plus frais. On a rencontré des différences significatives dans la teneur en 2-méthoxy-3-isobutylpyrazine entre les différentes variétés: Cabernet Sauvignon>Merlot>Syrah>Cencibel, qui ont été corroborées par la note attribuée par les dégustateurs au descripteur « arôme de poivron vert ». La corrélation entre le contenu en 2-méthoxy-3-isobutylpyrazine et ce descripteur suggère un seuil de perception à 3 ng/L dans les vins jeunes élaborés à Castilla-La Mancha, ce qui est assez inférieur à celui obtenu par d'autres auteurs dans des régions au climat plus frais.

Key words: flavour, grape, green pepper, pyrazine

Mots-clés : arôme, poivron vert, pyrazine, raisin

INTRODUCTION

The contribution of methoxypyrazines to green pepper aroma in grapes, and in wine varieties such as Cabernet Sauvignon, Merlot and Sauvignon blanc, has been studied by many authors in the past (BAYONOVE *et al.*, 1975; AUGUSTYN *et al.*, 1982; HARRIS *et al.*, 1987). The presence of these compounds has been established as a characteristic of the varietal aroma of these varieties.

The compounds from this group identified in wines are 2-ethyl-3-methoxypyrazine, 2-isopropyl-3-methoxypyrazine, 2-sec-butyl-3-methoxypyrazine and 2-isobutyl-3-methoxypyrazine. 2-methoxy-3-isobutylpyrazine is responsible for green pepper aroma due to its low perception thresholds, between 1 and 2 ng/L in water (ALLEN and LACEY, 1998) and 10 ng/L in red wine (KOTSERIDIS *et al.*, 1998).

Final methoxypyrazine content in wine is influenced by the methods used during the different stages of wine production. It has been confirmed that 2-methoxy-3-isobutylpyrazine can be easily extracted from grape skins in the Cabernet-Sauvignon variety; this takes place during the first hours of maceration. The clarification of must from the Sauvignon blanc variety diminishes its concentration by half. In wine production processes involving the maceration of grape skins, its final concentration seems to be independent of the number and duration of over-pumping procedures, although wines made from pressed grapes have higher concentrations (ROUJOU DE BOUBÉE *et al.*, 2002). Despite all these considerations, it has been noted that the most important factor is its initial concentration in grapes.

The content of 2-methoxy-3-isobutylpyrazine in grapes is determined by different climatological and agronomic factors, the determinant being the degree of ripeness of the grape berries which reduces this level (LACEY *et al.*, 1991). When grapes of the Cabernet-Sauvignon variety begin veraison, concentrations of around 100 ng/L (ALLEN *et al.*, 1998; HASHIZUME *et al.*, 1999) have been detected and in ripe grapes, around 8-15 ng/L (ALLEN *et al.*, 1991; ROUJOU DE BOUBÉE *et al.*, 1999). These authors also draw a parallel between the decrease in 2-methoxy-3-isobutylpyrazine content and the degradation of malic acid with temperature, the scorching of grape bunches (HASHIZUME *et al.*, 1999; SALA *et al.*, 2004) or water contribution to the vine (TANDONNET *et al.*, 1996; SALA *et al.*, 2005).

This study aims to examine methoxypyrazine concentration in the four grapes varieties mentioned at ripeness, cultivated in a warm climate, as well as its sensory impact in the resulting wines.

MATERIALS AND METHODS

I - GRAPE SAMPLES AND WINES

Four (4) grape varieties were studied: Cabernet-Sauvignon, Cencibel, Merlot and Syrah. They were cultivated using the espalier system and pruned using the double cordon royat training system. The samples were taken from seven vineyards distributed throughout the Castilla-La Mancha area (Spain). The Cabernet Sauvignon variety was represented in 7 locations, Cencibel and Merlot in 6 and Syrah in 4, with a total of 23 samples.

The grape samples were close to technological ripeness (between 12.5 and 13.5 Baume). Contents of 2-methoxy-3-ethylpyrazine and 2-methoxy-3-isobutylpyrazine were determined by taking two samples per plot. Each sample was analysed separately and the mean was calculated.

The wines were prepared using grapes from the same sampling vineyards, at the temperatures and grape skin maceration times normally used in the region, and analysed sensorially before ageing.

II - GC/MS ANALYSIS OF METHOXYPYRAZINES.

The methyl, ethyl and isobutyl-methoxypyrazine (Aldrich) standards and the extraction solvents (Merck) used were of analysis quality. They were quantified by optimising an analysis method in grapes using GC/MS in accordance with the method published by Kotseridis *et al.* (1998), with various modifications.

III - EXTRACTION AND CONCENTRATION

1,000 g of grapes were crushed, adding 300 µL of 60 µg/L 2-methoxy-3-methyl pyrazine (final concentration, 18 ng/kg of grape) as internal standard. The crushed grapes were centrifuged at 3,000 g for 20 minutes. The resulting must was subjected to liquid-liquid extraction for 24 hours with 250 mL of pentane-dichloromethane 60:40 mixture. The organic extract was concentrated to 2 mL by distillation using a Vigreux distilling column and stored at -20 °C until it was analysed.

IV - ANALYSIS.

The samples were analysed in a Trace2000 gas chromatograph coupled to a TraceMS mass spectrometer with an electron-impact ionisation source and a quadrupole analyser, equipped with an AS2000 automatic injector, all by ThermoQuest. A BP21 polyethylene glycol (TPA-treated), FFAP-bonded column was used to perform chromatographic separation, measuring 50 m in length, 0.32 mm in internal diameter and 0.25 µm thick in the stationary phase, with the following programmed tem-

Table I - Basic data of the 2-methoxy-3-ethyl and 2-methoxy-3-isobutylpyrazine analysis method.

Données de base de la méthode d'analyse de 2-méthoxy-3-éthyl- et 2-méthoxy-3-isobutylpyrazine.

Compound	TR (min)	m/z quantification	m/z qualification	Calibration interval (ng/kg)	R2 calibrated
2-methoxy-3-methylpyrazine (I.S.)	32.37	124	106		
2-methoxy-3-ethylpyrazine	36.55	138	123	0.8-76.2	0.9995
2-methoxy-3-isobutylpyrazine	42.78	124	151	0.7-72.8	0.9998

I.S: internal standard.

peratures: 43 °C (15 min) - 2 °C/min - 125 °C - 30 °C/min - 200 °C (45 min). The sample (2 µL) was injected in the split/splitless injector at 210 °C, with a splitless time of 1 minute and a split flow of 50 mL/min. The carrier gas used was helium at 1.4 mL/min.

The key parameters of the mass detector were: detector voltage, 500 V; emission current, 150 mV; impact energy, 70 eV. The chromatograms were obtained in SIM mode with the quantification and qualification ions shown in table I.

Figure 1 shows the part of chromatogram containing the compounds studied and table I shows the retention time, quantification and qualification ions used and the basic calibration data. The quantification limit (10 times the base line noise), recovery and repeatability were also studied; the results of which are shown in table II. The same tests were also performed in wines but the results were not as good; this could be due to the fact that the extraction of the compounds of interest was hindered by the ethanol present in the medium and the presence of

greater interference in these samples, due to the formation of a large number of volatile compounds during fermentation.

V - SENSORY ANALYSIS

The Sensorial Profile method was applied and performed five months after the wines were bottled, by a panel of 7 previously trained tasters. The wines were analysed exclusively from the aromatic rather than gustative standpoint, with special attention to the green pepper descriptor. Scores of 0 to 10 were awarded according to the intensity of this aroma in wine: 0 = not perceptible; 10 = very intense.

VI - STATISTICAL ANALYSIS

The data obtained from the 23 grape samples of the four varieties were subjected to an analysis of variance and the Student-Newman-Keuls test to identify the existence of statistically significant differences between varieties. A regression study was also performed on pyrazine content and the intensity of green pepper aroma. All these statistical tests were performed using SPSS software, version 12.0.

RESULTS AND DISCUSSION

Table III shows the mean 2-methoxy-3-ethylpyrazine and 2-methoxy-3-isobutylpyrazine contents in the four varieties of grapes, as well as the mean score awarded by the panel of tasters to the corresponding wines in relation to « green pepper » aroma. The statistically significant differences between varieties are indicated by mean superscripts.

The analysis of variance did not reveal any significant difference for 2-methoxy-3-ethylpyrazine. Mean content was very similar in all four varieties, around 3 ng/kg of grape clearly below the detection threshold of 425 ng/L.

2-methoxy-3-isobutylpyrazine content in the grapes was between 1.7 and 4.5 ng/kg. In this case, statistically significant differences were observed, Cabernet-Sauvignon grapes being the variety containing the largest amount of

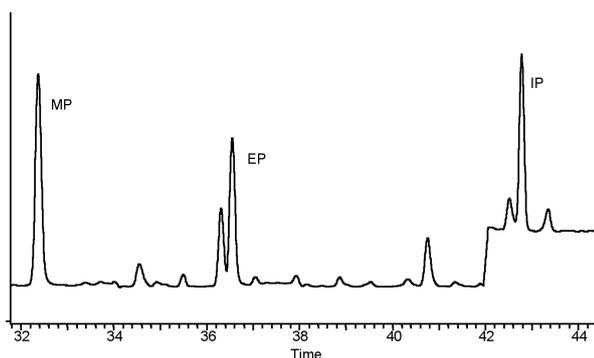


Figure 1 - Chromatogram of Cabernet-Sauvignon grape.

MP: 2-methoxy-3-methylpyrazine (internal standard), EP: 2-methoxy-3-ethylpyrazine, IP: 2-methoxy-3-isobutylpyrazine. Concentrations: 5 ng/kg of EP and 8 ng/kg of IP

Chromatogramme de raisin Cabernet-Sauvignon.

MP : 2-méthoxy-3-méthylpyrazine (étalon interne), EP : 2-méthoxy-3-éthylpyrazine, IP : 2-méthoxy-3-isobutylpyrazine. Concentrations : 5 ng/kg EP et 8 ng/kg IP.

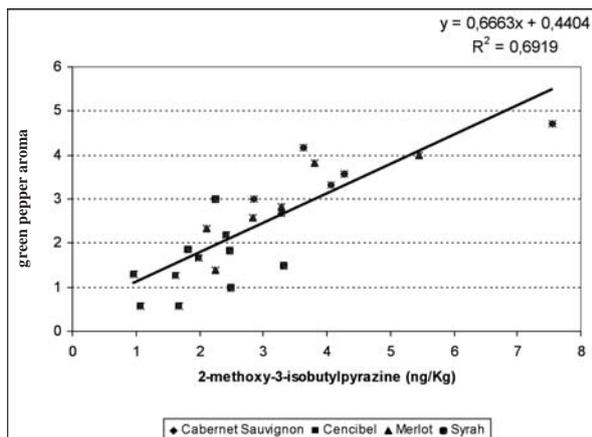


Figure 2 - Correlation between 2-methoxy-3-isobutylpyrazine content in the grapes and the green pepper aroma score of the wines

Corrélation entre le contenu en 2-méthoxy-3-isobutylpyrazine dans le raisin et la note du descripteur arôme poivron vert des vins

this compound (4.52 ng/kg of grape) and Cencibel the smallest amount (1.74 ng/kg of grape). These concentrations were lower than those reported by other authors for musts of Cabernet and Merlot varieties cultivated in cooler areas (15-20 ng/L (ALLEN *et al.*, 1999; ROUJOU DE BOUBÉE *et al.*, 1999)), confirming the influence of temperature during ripening reported in different studies.

The mean score awarded by the tasters for green pepper aroma also reflected significant differences similar to those observed in the chemical analysis: this sensory attribute was most intense in the Cabernet-Sauvignon wines and least intense in the Cencibel wines.

Figure 2 shows the straight line correlating 2-methoxy-3-isobutylpyrazine content in the grapes and the mean score for the green pepper descriptor awarded by the panel of tasters to the corresponding wines. Roujou de Boubée considered that the determination of the threshold for detecting a compound in wines cannot be a single, universal value given the huge variation in composition that may exist between each processing method, grape variety, etc., and established, for Bordeaux wines aged for dif-

ferent periods in oak barrels, an 2-methoxy-3-isobutylpyrazine detection threshold of 15 ng/L. In our young wines (before ageing in wooden barrels), the tasters perceived a weakly green pepper flavour (minimum score of 2 points) when the 2-methoxy-3-isobutylpyrazine concentration in grape was above 3 ng/Kg.

Figure 2 suggests that, in our study, the detection threshold of wines prepared using the method traditionally employed in our region, and before ageing in wooden barrels (young wines), may be established as from 3 ng/L, since the tasters awarded a minimum score of 2 points to wines with concentrations above that threshold (classified as « weakly perceptible »).

CONCLUSIONS

This is the first study to examine ethyl and 2-methoxy-3-isobutylpyrazine content in Cencibel grapes cultivated in a warm climate. The concentrations of 2-methoxy-3-isobutylpyrazine found were lower than those of Cabernet Sauvignon grapes and were not detected sensorially. These results seem to indicate that the sensory differences observed in relation to green pepper aroma were quantitative rather than qualitative, because in the four varieties analysed the two methoxy-pyrazines were found.

The concentrations of 2-methoxy-3-isobutylpyrazine detected in Cabernet-Sauvignon and Merlot grapes (the most studied in this field) cultivated in our region, with its characteristic warm climate in summer, displayed net lower concentrations than those reported by other authors in studies performed in regions with cooler climates, and this fact may have a favourable impact on their sensory characteristics.

Acknowledgements : This study was carried out thanks to funding from the Department of Science and Technology of the JCCM (Regional Government of Castilla-La Mancha) (Project PCC-02-003).

Raquel Romero Peces would like to thank the Instituto de la Vid y el Vino de Castilla-La Mancha for her the grant.

Table II - Results of the quantification test of the analysis method
Résultats des tests de quantification de la méthode d'analyse

Compound	Quantification limit (ng/kg)	Standard additions interval (μ g/L)	Recovery (%)	Repeatability SD R (%) (n=10)
2-methoxy-3-ethylpyrazine	1.52	1.5-30.5	95-109	5.1
2-methoxy-3-isobutylpyrazine	1.73	1.5-29.1	98-106	4.8

Table III - Concentrations of 2-methoxy-3-ethyl and 2-methoxy-3-isobutylpyrazine and mean scores of « green pepper » aroma

Concentration des 2-méthoxy-3-ethyl et 2-méthoxy-3-isobutylpyrazine et notes moyennes de l'arôme de poivron vert

Variety	C. Sauvignon		Cencibel		Merlot		Syrah	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
2-methoxy-3-ethyl-pyrazine	3.30	1.00	3.93	0.72	3.04	1.06	3.59	0.68
2-methoxy-3-isobutyl-pyrazine	4.52 ^a	1.79	1.74 ^b	0.62	3.12 ^{ab}	1.31	2.47 ^b	0.63
Green Pepper Descriptor	3.58 ^a	0.84	1.25 ^c	0.70	2.83 ^{ab}	1.09	1.84 ^{bc}	0.85

Different superscripts (^{a,b,c}) indicate the existence of statistically significant differences according to the Student-Newman-Keuls multiple comparison test. $\alpha=0.05$

REFERENCES

- ALLEN M.S. and LACEY M.J., 1993. Methoxypyrazine grape flavour: influence of climate, cultivar and viticulture. *Vitic. Enol. Sci.*, **48**, 211-213.
- ALLEN M.S. and LACEY M.J., 1998. Methoxypyrazines of grapes and wines. In: Chemistry of wine flavour. ACS Symposium Series 719. American Chemical Society, Washington D.C. Eds. A.L. Waterhouse and S.E. Ebeler. Chapter 3.
- AUGUSTYN O.P.H., RAPP A. and VAN WYCK C.J., 1982. Some volatile aroma components of *Vitis vinifera* L. cv. Sauvignon blanc. *S. Afr. J. Enol. Vitic.*, **3**, 53-60.
- BAYONOVE C., CORDONNIER R.A. and DUBOIS P., 1975. Etude d'une fraction caractéristique de l'arôme du raisin de la variété Cabernet-Sauvignon; mise en évidence de la 2-methoxy-3-isobutylpyrazine. *C.R. Acad. Sci. (Paris), Série D*, **281**, 75-78.
- BLOUIN J. and GUIMBERTEAU., 2000. *Maturation et maturité*. Ed. Féret. Bordeaux.
- HARRIS R.L.N., LACEY M.J.; BROWN W.V. and ALLEN M.S., 1987. Determination of 2-methoxy-3-alkylpyrazines in wines by gas chromatography/mass spectrometry. *Vitis*, **26**, 201-207.
- HASHIZUME K. and SAMUTA T., 1999. Grape maturity and light exposure affect berry methoxypyrazine concentration. *Am. J. Enol. Vitic.*, **50**, 194-198.
- KOTSERIDIS Y., ANOCIBAR BELOQUI A., BERTRAND A. and DOAZAN J.P., 1998. An analytical method for studying the volatile compounds of Merlot noir clone wines. *Am. J. Enol. Vitic.*, **49**, 44-48.
- LACEY M.J., ALLEN M.S., HARRIS R.L.N. and BROWN W.V., 1991. Methoxypyrazines in Sauvignon blanc grapes and wine. *Am. J. Enol. Vitic.*, **42**, 103-108.
- ROUJOU DE BOUBÉÉ D. and DUBOURDIEU D., 1999. Incidence des conditions de maturation et des pratiques viticoles sur la teneur en 2-methoxy-3-isobutylpyrazine des raisins de Cabernet Sauvignon et de Merlot à Bordeaux. In: *Oenologie 99, 6^e Symp. Int. CEnol.* Lonvaud and Funel Ed. Tec&Doc. pp126-130.
- ROUJOU DE BOUBÉÉ D., CUMSILLE A.M., PONS M. and DUBOURDIEU D., 2002. Location of 2-methoxy-3-isobutylpyrazine in Cabernet-Sauvignon grape bunches and its extractability during vinification. *Am. J. Enol. Vitic.* **53**, 1-5.
- SALA C., BUSTO O., GUASCH J. and ZAMORA F., 2005. Contents of 3-alkyl-2-methoxypyrazines in musts and wines from *Vitis vinifera* variety Cabernet Sauvignon: influence of irrigation and plantation density. *J. Sci. Food Agric.*, **85**, 1131-1136.
- SALA C., ZAMORA F., BUSTO O., GUASCH J.S., 2004. Influence of vine training and sunlight exposure on the 3-alkyl-2-methoxypyrazines content in musts and wines from the *Vitis vinifera* variety Cabernet-Sauvignon. *J. Agric. Food Chem.* **52**, 3492-3497.
- TANDONNET J.P., OLLAT N., NEVEUX M. and RENOUX J.L., 1996. Effect of three levels of water supply on the vegetative and reproductive development of Merlot and Cabernet Sauvignon grapevines. In: Proceedings of 1st ISHS Workshop on water relations of grapevines. Ruhl E.H. and Schmid J. Eds., *Acta Hort.*, **493**, 301-307.

Manuscrit reçu le 16 novembre 2005 ; accepté pour publication, après modifications le 14 novembre 2006