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Contents of 3-alkyl-2-methoxypyrazines in musts and wines from *Vitis vinifera* variety Cabernet Sauvignon: influence of irrigation and plantation density

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Abstract: The influence of irrigation and plantation density on the methoxypyrazine content in musts and wines has been studied. Samples were monitored throughout grape ripening and wine-making. 3-Isobutyl-2-methoxypyrazine, 3-*sec*-butyl-2-methoxypyrazine and 3-isopropyl-2-methoxypyrazine were identified and quantified. Samples from irrigated vines had significantly higher average contents of 3-isobutyl-2-methoxypyrazine than samples from non-irrigated plants. Average levels of this compound were also higher in samples from vines with the higher plantation density.

Keywords: 3-alkyl-2-methoxypyrazines; Cabernet Sauvignon; grape; irrigation; plantation density; wine

INTRODUCTION

Trace amounts of 3-alkyl-2-methoxypyrazines (MPs) can be found in a wide range of vegetables, eg peas and bell peppers.[1,2] These substances are important flavor compounds due to their extremely low sensory detection thresholds. 3-Isobutyl-2-methoxypyrazine (IBMP) is perceptible at 0.5–2 ng l^{-1} in water, synthetic wine and white wine, and at 10–16 ng l^{-1} in red wine. 3-*sec*-Butyl-2-methoxypyrazine (SBMP) can be detected by the human nose at 1 ng l^{-1} in water, and the sensory detection threshold of 3-isopropyl-2-methoxypyrazine (IPMP) in water is 2 ng l^{-1}.[3–9] These three compounds have been associated with the green, herbaceous or vegetative aromas that are characteristic of Cabernet Sauvignon, Cabernet Franc, Sauvignon blanc and Merlot wines.[6,10–17] They can occur in berries and wines of these varieties at levels higher than their sensory detection thresholds and have an important impact on wine quality.

The 'vegetative' aromas found in red wines containing IBMP are generally considered unfavourable to wine quality.[6,18] However, the presence of this compound at relatively high levels can be compatible with the high quality of red wines,[19,20] as long as it is not too dominant and it is complemented by other aromas.[6,21]

Different grape cultivars have been proven to contain diverse amounts of MPs, suggesting that these compounds can contribute to their varietal distinction. IBMP levels in Merlot samples were lower than those in Cabernet Sauvignon wines of the same vintage,[20] and important differences in MP contents in grapes between the varieties Cabernet Sauvignon, Merlot, Semillon, Sauvignon blanc and Riesling have been reported.[22] MPs are part of the characteristic aromas of the variety Sauvignon Blanc, together with other aromas, among which the sulfur-containing 4-mercapto-4-methyl-pentan-2-one and 3-mercapto-hexan-1-ol are also important contributors.[23,24]

Several reports on how viticultural factors may influence the MP contents in grapes and wines have been published,[10,17,20–22] but information about how plantation density and irrigation can affect the amounts of these compounds in grapes and wines is rather scarce.

Climatic conditions during grape ripening, particularly at the unripe grape stage, may determine IBMP contents in final wines.[22] A higher humidity in the preveraison month may result in higher IBMP contents in the grapes at harvest: a sunnier and less humid year led to lower IBMP amounts, whereas levels of this compound were clearly higher in the following year,

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which was marked by frequent rainfall. The authors explained that the reconstitution of soil water reserves favored the growth of the vine until harvest which, in turn, increased the production and retention of IBMP.[18]

It has been reported that there is an association between the 'vegetative' notes of wines and the deep, clay-rich soils that are nutrient-rich and have a high water-holding capacity. On the other hand, fruitier wines, richer in berry aromas, have been linked to shallow, sandy soils that are nutrient-poor and have a lower water-holding capacity. The authors attributed this difference to the fact that the first type of soils produces more vigorous canopies, which limit the sunlight exposure of the berry, whereas vines grown in the latter type of soils produce a wide, open canopy, the fruits of which are better exposed to sunlight.[25]

Cabernet Sauvignon grapes from vineyards with different plantation densities and in soils with different compositions showed different evolution of IBMP contents throughout the grape-ripening process.[18] Unfortunately, the effect of soil could not be separated from the effect of plantation density, so that further research is needed to understand to what extent each factor contributes to the differences in MP contents.

Grape growers and wine-makers are interested to know how viticultural factors may influence MP contents in grapes and how these compounds pass to final wines, in order for them to manage and obtain the maximum wine quality. With the aim of enlarging the data available in this field, the present article focuses on the study of how irrigation and plantation density may affect the contents and evolution of MPs in grapes and wines. Analysis were performed by means of two fast, in expensive and convenient methods based on HS-SPME and GC-NPD, which we developed previously.[26,27]

EXPERIMENTAL

All samples were produced and collected at the experimental fields and cellar of the Facultat d'Enologia de Tarragona (Universitat Rovira i Virgili) at Constantí (Tarragona) in 1998. The weather in the region (average June, July and August, respectively) was as follows: temperature, 20.6, 23.6 and 23.5 °C; maximum temperature, 26.1, 29.2 and 29.9 °C; minimum temperature, 15.2, 17.8 and 17.6 °C; daily solar irradiation, 21.7, 22.6 and 17.2 $MJ\,m^{-2}$; rainfall, 1.0, 3.5 and 35.0 $l\,m^{-2}$.

Vines

This study was carried out with the Cabernet Sauvignon variety. The clone used was number 15 of Rauscedo that was planted on rootstock 110 Richter. All vines involved in this study were trained in a bilateral cordon (Cordon de Royat double) and trellised in a spalier system with three levels (60, 110 and 150 cm). After pruning the vines had 16 buds per plant.

Samples for the study of irrigation were collected from a vineyard that had a total of 480 plants and a plantation density of 2500 plants per hectare (40 000 buds per hectare). The plot was divided into 16 subplots, each one containing five rows of six plants per row: five vines were Cabernet Sauvignon, and the sixth one, of another variety, was used to prevent possible interferences between treatments. There were four groups of four subplots, alternately distributed. They received four different irrigation treatments, although only the two extremes, non-irrigated and irrigated, were used in this study. Irrigation was achieved by means of a trickle system. Irrigated vines received 6 $l\,h^{-1}$ for a total of 4 h per week, during 6 weeks all through grape ripening.

Samples for the study of plantation density were collected from an experimental plot that had a total of 270 plants. It was not irrigated. The plot was divided alternately into nine subplots and there were three groups of three subplots, with the following plantation densities: 2000, 3000 and 4000 plants per hectare (32 000, 48 000 and 64 000 buds per hectare).

Sampling

In order to obtain random samples and not to repeat the same vine in the different sampling times, a mark was put on every fifth vine of each vineyard. The first sample was collected only from the marked vines. The second sample was collected from the vine immediately next to the marked vine. The third to fifth samples were collected from the plants on the third to fifth places after the marked vine.

Grapes for sampling were also randomly selected within the vine, so that a homogeneous distribution between those more and less exposed to sunshine was ensured.

Three berries from each grape were collected up to a total of about 100. They were randomly sampled, taking one from the top, one from the bottom, and one from the middle of the cluster. Special care was taken in order to obtain a good distribution between berries from the inner and outer parts of the cluster. Three replicates of each kind of sample were collected in all cases.

The first sample was taken at veraison and the last one at harvest. Three intermediate samples were collected throughout grape ripening. After removing the bunchstems, grapes were manually pressed at the laboratory and 1 $g\,l^{-1}$ of NaF was added to the grape juice to preserve it. Samples were kept in dark bottles at −20 °C until analysis. Classical red wine-making was followed in all types of samples. Musts were fermented in pilot-scale tanks. Sampling times over wine-making were the following: musts after one day of maceration, and at the end of alcoholic fermentation and final wines at the end of malolactic fermentation. Three replicates of each sample were taken and analysed in all cases, each sample belonging to a different fermentation tank. Fermented samples were preserved with SO_2 and kept in dark bottles at 4 °C until analysis.

Analytical procedures

Analysis of samples was performed according to the previously published HS-SPME procedures.[26,27] Every determination was made in duplicate. Chromatographic analysis was performed with a Hewlett-Packard (Palo Alto, CA, USA) 5890 II gas chromatograph equipped with a nitrogen-phosphorous detector (NPD). Injection (splitless, 1 min) was performed with an inlet of 0.75 mm i.d. and at 250 °C. The analytical column was a CP-WAX 57 CB (50 m × 0.25 mm id, 0.2 μm film thickness). The carrier gas was high-purity helium flowing at 0.8 ml min^{-1}. Oven temperature was 30 °C (1 min), 25 °C min^{-1} to 100 °C (20 min). 3-Isopropyl-2-ethoxy-pyrazine was used as internal standard, and was provided by Pyrazine Specialties (Atlanta, GA, USA), its purity being above 97%. SPME device and polydimethylsiloxane/divinylbenzene (65 μm) fibers used in this study were purchased from Supelco (Bellefonte, PA, USA). Each fiber was conditioned before use as well as cleaned after use by inserting them into a GC injector at 260 °C for minimum of 5 min. They were immediately used to prevent contamination. Results were statistically analyzed by means of two-factor ANOVA and Fisher test ($p = 0.05$ for both).

Statistics

All the data are expressed as the arithmetic average ± standard deviation from three replicates. Two-factor ANOVA and Fisher test were carried out using Statview.[28]

RESULTS

MP contents in experimental musts and wines are shown in Tables 1–7. Figures 1 and 2 show two typical chromatograms obtained with the grape juices and wines analyzed. IBMP, SBMP and IPMP were identified. 3-Ethyl-2-methoxypyrazine was found in some samples, but its contents were usually below quantification limits. Generally, IBMP was the most abundant MP and concentrations of SBMP were higher than IPMP concentration. IBMP levels decreased significantly throughout grape ripening in all monitored plots, so that the lowest concentrations are reached at harvest. The process of alcoholic fermentation/maceration with the solid parts of the berries raised the IBMP contents in all samples analysed, particularly within the first day of maceration (Tables 2 and 6).

At veraison, samples from irrigated and non-irrigated vines did not show important differences on IBMP contents (Table 1). This is logical because veraison took place only a few days after starting

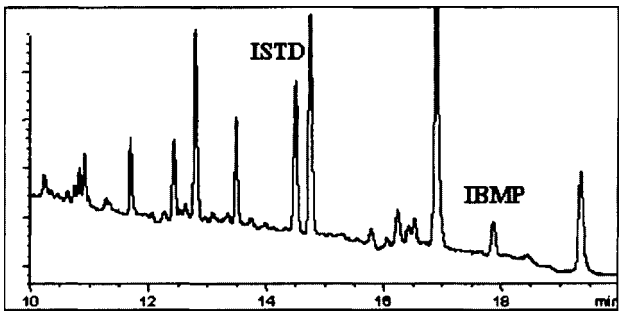

Figure 1. Example of a chromatogram of a must. Internal standard (ISTD): 3-isopropyl-ethoxypyrazine (10 ng l^{-1}).

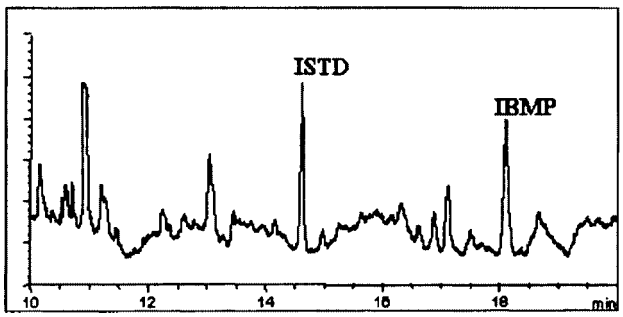

Figure 2. Example of a chromatogram of a wine. Internal standard (ISTD): 3-isopropyl-ethoxypyrazine (10 ng l^{-1}).

Table 2. IBMP contents (ng l^{-1}) throughout wine-making. Irrigation effect

	Non-irrigated	Irrigated
Harvest	—[a]	3.9 (2.0) A
1 day maceration	4.3 (1.9) a	6.1 (1.3) B
End alcoholic fermentation	8.3 (3.3) a	13.1 (3.2) C
End malolactic fermentation	9.6 (3.8) a	15.3 (4.1) C

Two-factor ANOVA: wine-making, $p < 0.0001$; treatment, $p = 0.0067$; interaction, $p = 0.6477$.
[a] Below quantification limits. Values followed by a different letter are significantly different at the $p < 0.05$ level.

Table 1. IBMP contents (ng l^{-1}) throughout grape ripening. Irrigation effect

		Non-irrigated		Irrigated	
	Date	Sugar (g l^{-1})	IBMP (ng l^{-1})	Sugar (g l^{-1})	IBMP (ng l^{-1})
Veraison	11 August	133	34.2 (3.6) a	131	38.0 (6.4) A
Ripening 1	24 August	160	16.1 (8.8) b	159	20.0 (8.4) B
Ripening 2	3 September	182	5.6 (3.4) c	190	17.3 (7.2) B
Ripening 3	10 September	192	4.4 (0.4) c	199	6.1 (3.3) C
Harvest	16 September	207	—[a]	206	3.9 (2.0) C

Two-factor ANOVA: ripening, $p < 0.0001$; treatment, $p = 0.0306$; interaction, $p = 0.5538$.
[a] Below quantification limits. Values followed by a different letter are significantly different at the $p < 0.05$ level.

Table 3. SBMP contents (ng l^{-1}) throughout grape ripening. Irrigation effect

		Non-irrigated		Irrigated	
	Date	Sugar (g l^{-1})	SBMP (ng l^{-1})	Sugar (g l^{-1})	SBMP (ng l^{-1})
Veraison	08–11	133	4.9 (1.4) a	131	5.8 (2.4) A
Ripening 1	08–24	160	2.6 (0.5) a	159	10.2 (4.7) AB
Ripening 2	09–03	182	5.8 (2.9) a	190	16.1 (7.5) B
Ripening 3	09–10	192	2.8 (1.3) a	199	4.6 (2.5) A
Harvest	09–16	207	— [a]	206	3.3 (1.1) A

Two-factor ANOVA: ripening, $p = 0.0035$; treatment, $p = 0.0015$; interaction, $p = 0.0908$.

[a] Below quantification limits. Values followed by a different letter are significantly different at the $p < 0.05$ level.

the irrigation treatment and, therefore, no differences were expected at this stage. Afterwards, in both kinds of grapes, levels of this compound dropped throughout grape ripening. However, the decrease in concentration in grapes from irrigated plants happened later. At harvest, IBMP contents were low in both cases, although only in the samples from irrigated vines were they high enough to be determined. Finally, wines from irrigated vines contained significantly higher levels of IBMP (Table 2). Similarly, SBMP contents in grapes from irrigated vines were significantly higher (Table 3). Also, although statistical analysis could not be performed for IPMP, the contents of this compound also tended to be higher in grapes from the irrigated plants (Table 4). The contents of SBMP and IPMP throughout wine-making are not presented because they were close to or below quantification limits all through this process and none of these compounds were detected in final wines.

Over the grape-ripening period, contents of IBMP and SBMP were significantly higher in samples from vines with the highest plantation density (Tables 5 and 7). Levels of SBMP were low over the grape ripening period and did not show any clear increase or decrease except that, at harvest, they were below quantification levels in all cases (Table 7). IBMP contents were raised during wine-making (Table 6), but not to the levels of SBMP or IPMP, which remained close to or below quantification limits until the end of the process. Final wines made with grapes from the highest plantation density vines contained significantly higher contents of IBMP (Table 6). IPMP levels were between 2.8 and 5.9 (with no significant differences over the process) in samples from vines with a plantation density of 4000 plants per hectare throughout wine-making. However, they were below quantification limits in samples from both 3000 and 2000 plants per hectare vines.

DISCUSSION AND CONCLUSIONS

Concentrations of IBMP in final wines (4.6–15.5 ng l^{-1}; Tables 2 and 6) match the literature: it has been reported that red wines contain 3.6–56.3 ng l^{-1} of this compound.[6,11,20] The fact that IBMP contents

Table 4. IPMP contents (ng l^{-1}) throughout grape ripening. Irrigation effect

		Non-irrigated		Irrigated	
	Date	Sugar (g l^{-1})	IPMP (ng l^{-1})	Sugar (g l^{-1})	IPMP (ng l^{-1})
Veraison	8 August	133	2.6 (0.5) a	131	4.3 (0.9) A
Ripening 1	24 August	160	— [a]	159	4.9 (1.5) A
Ripening 2	3 September	182	3.4 (1.2) a	190	15.0 (7.1) B
Ripening 3	10 September	192	— [a]	199	2.7 (0.6) A
Harvest	16 September	207	— [a]	206	— [a]

[a] Below quantification limits. Values followed by a different letter are significantly different at the $p < 0.05$ level. Results could not be analysed by means of two-factor ANOVA test due to the lack of data above quantification limits.

Table 5. IBMP contents (ng l^{-1}) throughout grape ripening. Effect of plantation density (plants per hectare)

		2000		3000		4000	
	Date	Sugar (g l^{-1})	IBMP (ng l^{-1})	Sugar (g l^{-1})	IBMP (ng l^{-1})	Sugar (g l^{-1})	IBMP (ng l^{-1})
Veraison	12 August	128	32.4 (7.4) a	129	28.0 (7.5) A	129	42.5 (8.6) α
Ripening 1	26 August	162	10.9 (3.8) b	158	13.7 (5.8) B	163	15.6 (5.3) β
Ripening 2	5 September	185	5.7 (2.9) c	192	7.4 (6.3) BC	189	10.7 (3.3) β
Ripening 3	10 September	201	5.8 (2.3) c	199	4.0 (2.0) C	199	7.0 (2.9) β
Harvest	17 September	216	— [a]	214	— [a]	218	2.6 (0.4) γ

Two-factor ANOVA: ripening, $p < 0.0001$; treatment, $p = 0.0267$; interaction, $p = 0.4084$.

	2000	3000
3000	0.8468	—
4000	0.0237	0.0153

[a] Below quantification limits. Values followed by a different letter are significantly different at the $p < 0.05$ level.

Table 6. IBMP contents (ng l^{-1}) throughout wine-making. Effect of plantation density (plants per hectare)

	2000	3000	4000
Harvest	— [a]	— [a]	2.6 (0.4) α
1 day maceration	6.7 (3.4) a	6.4 (0.9) A	9.5 (3.6) β
End of alcoholic fermentation	11.6 (3.9) a	10.8 (3.2) B	14.8 (3.2) $\beta\gamma$
End of malolactic fermentation	9.8 (3.4) a	9.9 (2.9) B	15.5 (2.4) γ

Two-factor ANOVA: wine-making, $p = 0.0341$; treatment, $p = 0.0463$; interaction, $p = 0.9533$.

	2000	3000
3000	0.9196	—
4000	0.0279	0.0347

[a] Below quantification limits. Values followed by a different letter are significantly different at the $p < 0.05$ level.

Table 7. SBMP contents (ng l^{-1}) throughout grape ripening. Effect of plantation density (plants per hectare)

		2000		3000		4000	
	Date	Sugar (g l^{-1})	SBMP (ng l^{-1})	Sugar (g l^{-1})	SBMP (ng l^{-1})	Sugar (g l^{-1})	SBMP (ng l^{-1})
Veraison	12 August	128	4.3 (1.3) a	129	4.0 (2.0) A	129	7.4 (3.1) α
Ripening 1	26 August	162	2.5 (0.3) a	158	4.9 (2.1) A	163	4.6 (2.2) α
Ripening 2	5 September	185	3.0 (0.6) a	192	4.1 (0.9) A	189	6.4 (1.6) α
Ripening 3	10 September	201	3.9 (1.2) a	199	3.5 (2.0) A	199	3.6 (1.4) α
Harvest	17 September	216	— [a]	214	— [a]	218	— [a]

Two-factor ANOVA: ripening, $p = 0.0634$; treatment $p = 0.0457$; interaction, $p = 0.3861$.

	2000	3000
3000	0.3346	—
4000	0.0142	0.1097

[a] Below quantification limits. Values followed by a different letter are significantly different at the $p < 0.05$ level.

are generally higher than the levels of both SBMP and IBMP can also be found in the literature.[3,11,15–17] SBMP and IPMP contents in final wines, close to or below quantification limits, also agree with the reported studies: 0.05–1.9[11] and 0.92–10.1 ng l^{-1},[12] respectively, in red wines. Taking into account the sensory thresholds mentioned in the introduction, results reveal that IBMP is the MP more likely to influence the flavor of the final wines. Our findings agree with previous studies.[12,20,22] Therefore, in terms of sensory impact, this compound can be considered the most important methoxypyrazine in the studied Cabernet Sauvignon samples.

The decrease in IBMP contents over grape ripening (Tables 1 and 5) agrees with the literature.[16,17,20,21] Results also show that such decrease happens mainly at the first stage of the ripening process, as previously reported.[10,17,26,27] This means that MP contents in final wines can be determined by the harvest date. Therefore, the analytical quantification of MPs in grapes, together with a further knowledge on how MPs pass to wines, may be useful to determine the suitable grape maturity conditions for producing high-quality wines.

References to the observed rise in IBMP contents throughout wine-making (Tables 2 and 6) can also be found in the literature.[29] However, no major changes in MP concentrations were observed after racking. As discussed in a previous work,[27] all these results point to the hypothesis that MPs pass to the juice during the wine-making process because they are partly located at the skins, seeds and stems of the fruits.[16,20,22,30] The rise in MP contents happens mainly at the first day after destemming and crushing the grapes, before starting the alcoholic fermentation. Therefore, it is probable that the highest contact between skin/seeds/stems and juice after crushing the grapes is the factor that accelerates the pass of MPs from the solid parts to the musts. On account of this, it is possible that the duration of maceration may influence the levels of MPs in the final wines.[20]

The observed delay in the drop of IBMP levels of the grapes belonging to the irrigated plants (Table 1) can be due to the fact that these fruits ripen at a slightly slower rhythm, although they reach harvest with the same sugar level. Plantation density seems to be a factor that can condition IBMP levels in grapes and wines (Tables 5 and 6), since it has been observed that averages show a tendency to be significantly higher in samples from vines with the highest plantation density in all cases. Finally, considering the sensory detection threshold of IBMP,[6] samples from irrigated vines and also from vines with a higher plantation density are more likely to have the characteristic 'vegetative' flavor.

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