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Quantitative determination of 2-methoxy-3-isobutylpyrazine in red wines and grapes of Bordeaux using a stable isotope dilution assay

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Abstract

Quantitative analysis of 2-methoxy-3-isobutylpyrazine (MIBP) in grapes and wines was developed, using a stable isotope dilution assay. This was applied to red grapes and wines from the Bordeaux region. The grapes and the wines of the 1995 and 1996 vintages came from the three most frequently used varieties of the region, Merlot, Cabernet Franc and Cabernet Sauvignon. The wines made from Cabernet Sauvignon grapes exhibited levels of MIBP (mean concentration, 12 ng l^{-1} for 1996 vintage and 13 ng l^{-1} for 1995 vintage) close to or higher than its odour threshold in wines (10 ng l^{-1}) and slightly higher than the amounts found in the Merlot wines (mean concentration, 8 ng l^{-1} for 1996 vintage and 4 ng l^{-1} for 1995 vintage), especially those of the 1996 vintage. The variation in the levels of MIBP in grape samples and in their corresponding wines was monitored at four different stages towards the end of maturation. MIBP was present in all grapes and wines analysed, even in surmaturation. A linear trend was observed between grapes and wines of the three cultivars during maturation.

Keywords: Grapes; Fruits; Wine; Food analysis; Methoxyisobutylpyrazine; Pyrazines

1. Introduction

One of the main 'varietal' volatile odorants of gape and wine, is 2-methoxy-3-isobutylpyrazine (MIBP). MIBP was first identified in Cabernet Sauvignon grapes, by Bayonove et al. in 1975 [1] and later found in wines of Sauvignon blanc [2]. It has a potent odorant reminiscent of green bell peppers and contributes to the characteristic aroma of many vegetables, such as peas and bell peppers [3,4]. Its odour threshold value in water was first reported at 2 ng l^{-1} [5], however, it was recently suggested to be as low as 0.5 ng l^{-1} [6].

During the last decade, several methods were developed to determine MIBP quantitatively in grapes and wines. Quantification of this compound was attempted in a Chenin blanc wine using steam distillation followed by concentration on a C_{18} cartridge and high-performance liquid chromatography (HPLC), however, the recovery was low (52.9±7%) and the detection level was high (1.2 μg

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l^{-1}) [7]. Later, Boison and Tomlinson [8] used smaller quantities of wine (95 ml) and a specialised apparatus that was modified for solvent extraction. The results obtained indicated that there was an unusually high level (500 ± 70 ng l^{-1}) of MIBP in a Bordeaux wine and this is believed to be attributable to the acetophenone that was used as an internal standard. Acetophenone is a compound that is chemically completely different to MIBP.

Quantification of MIBP in wines, with accuracy and a detection limit below its odour threshold, was achieved in 1987 [9], using a stable isotope dilution assay, probably for the first time in wine aroma analysis. However, the laborious procedure of the isolation step, involving distillation and the use of a strong cation-exchange resin, could induce the partial loss of this trace compound. Levels of MIBP, varying between 3.6 and 56.3 ng l^{-1} in red wines, were reported [10], and these were similar to those previously reported [11] in Sauvignon blanc wines.

It has been shown that quantification of MIBP in wines is possible without using a deuterium-labelled internal standard [12]. The isolation step is the same as that reported previously [11] but was slightly modified to improve the recovery and detection limits. Similarly, Kotseridis et al. [6] reported an easy and quick isolation procedure for the analysis of MIBP in Merlot Noir wines, using 2-methoxy-3-methylpyrazine as an internal standard.

A two-step synthesis of labelled MIBP was also reported, starting from unlabelled MIBP and was used to study the impact odorants of coffee brews [13]. Nevertheless, the use of diazomethane in this synthesis requires a special apparatus, due to its explosive potential. Recently, Kotseridis et al. [14] developed an easy one-step synthesis of labelled MIBP and an improved method of isolation of this important odorant for determining its trace levels in a Merlot wine.

Levels of MIBP have been monitored in grapes during maturation. Lacey et al. [11] have shown that the concentration of MIBP in grape juices decreases during maturation. More than 96% of the veraison MIBP level was lost, at the moment of technological maturity, although the results presented later [15] were contradictory. The last three measurements of MIBP in Sauvignon blanc grapes during maturation indicated an increase in levels.

The aim of this investigation was to develop a rapid assay, using an easily synthesised deuterium-labelled MIBP [14] and a convenient method for the isolation of the target compound, applicable to the analysis of grapes and wines of different varieties, sourced from different Bordeaux (France) regions and at different stages of maturation.

2. Experimental

2.1. Materials

Wines: Twelve Merlot (six of the 1995 and six of the 1996 vintage), nine Cabernet Sauvignon (four of the 1995 and five of the of 1996 vintage) and four Cabernet Franc (two of the 1995 and two of the of 1996 vintage) wines from the Bordeaux regions of Margaux, Pauillac, Pomerol, Fronsac, Graves, Moulis and St. Emilon were analysed during this assay. All of the wines were sampled after malolactic fermentation was achieved, then they were drained and a mean amount of 40 mg l^{-1} SO_2 was added at bottling, before being stored at 10°C prior to analysis. The wines were between one and two years old at the time of analysis.

2.1.1. Maturation trials

Six vineyards were selected for this trial. The Merlot grapes were grown in Margaux (gravelly soil) and in Fronsac (clayey–chalky soil), the Cabernet Sauvignon grapes in Margaux (gravely soil) and in Pauillac (gravelly–sandy soil), the Cabernet franc grapes were grown in St. Emilon (clayey–chalky soil) and in St. Emilon Montagne (sandy soil). Bunches (70–80 kg per sampling) were harvested at regular intervals from a single row in each vineyard. Samples of 1000 berries were chosen at random for further analysis. The grapes were stored at −20°C until analysis. The remainder of the grapes were destemmed, crushed and put into 50 l stainless steel tanks. The must was treated with sulfur dioxide (50 mg l^{-1}), using a 6% (w/v) SO_2 solution. The musts were inoculated with dry active *Saccharomyces cerevisiae* yeast (Fermirouge, 0.2 g l^{-1}). The fermentation conditions were reported elsewhere [6]. Four samplings and four vinifications were made for each sample. In total, 24 samples of grapes and 24

samples of their corresponding wines were analysed during this trial.

2.2. Chemicals and other materials

MIBP was purchased from Aldrich (Milwaukee, WI, USA) and it had a purity of 99%. The compound was further purified using an Aldrich Short-Path Distillation apparatus with a jacketed distillation head. Detailed procedures for the synthesis of 2-methoxy-3-([1,1-$^2H_2$]isobutyl)pyrazine, are reported elsewhere [14]. The organic solvents, diethyl ether and hexane, were of ultra-pure grade and were obtained from SDS (Peypin, France). Cleaning of the glassware used for the isolation of MIBP from grapes and wines was performed using absolute alcohol and high purity water alternatively.

2.3. Classical grape and wine analysis

Total acidity was determined for wines and centrifuged juice samples by titrating them to pH 8.2 with 0.1 *M* NaOH in the presence of bromothymol blue (BBT). Determinations of the degrees of Brix (on the centrifuged juice), of ethanol (%, v/v, in wines) and pH were carried out. Determination of the content of anthocyanins (mg l^{-1}) in the grapes and the corresponding wines was carried out by measuring the absorbance at 520 nm [16].

2.4. Isolation of volatiles

Wine samples: A 250-ml volume of a wine sample was placed in a closed flask, then spiked with 500 μl of a solution containing 2-methoxy-3-([1,1-$^2H_2$]isobutyl)pyrazine in anhydrous alcohol (10 ng ml^{-1}) using a calibrated microliter syringe (SGE, 500 μl) and the mixture was stirred for 10 min (equilibration time) using a magnetic stirrer. The mixture was divided into two portions of 100 ml, placed in two 200 ml flasks, and each of them was extracted with 3×5 ml of diethyl ether–hexane (1:1, v/v) by stirring for 5 min with a magnetic stirrer (1100 rpm). The organic phases were separated in a separatory funnel, dried over Na_2SO_4, then filtered through glasswool and concentrated under a nitrogen (N_2, 5.0 quality) stream down to 100 μl. The final concentration factor was 1000.

Grape samples: Berry samples (about 1 kg each) were allowed to reach a temperature of 4°C overnight, were destemmed, crushed in a fruit-juicer for 2 min, then centrifuged (10600 *g*, 15 min), while keeping the temperature at 4°C. The juices were filtered through glasswool and the pH was adjusted to 8.8 with a 10-*M* NaOH solution. A 250-ml volume of the sample was spiked with 500 μl of a solution containing 2-methoxy-3-([1,1-$^2H_2$]isobutyl)pyrazine in anhydrous alcohol (10 ng ml^{-1}) and was thereafter analysed as described above.

2.4.1. Solvent choice for the recovery of MIBP from juices and wines

A Merlot wine was spiked with 60 ng l^{-1} of MIBP and was submitted to liquid–liquid extraction with solvents of different polarity: dichloromethane, diethyl ether, dichloromethane–pentane (1:2, v/v) and diethyl ether–hexane (1:1, v/v). After extraction, the organic phases were spiked with 250 μl of a solution containing [$^2H_2$]-MIBP in anhydrous alcohol (10 ng ml^{-1}), concentrated and analysed by gas chromatography–mass spectrometry (GC–MS). For the chosen solvent (diethyl ether–hexane), the extraction was carried out at the pH of the wine or grape as well as at a pH of 8.8; Furthermore, after the third extraction, the aqueous phase was extracted once more using 5 ml of the solvent, giving a total of four extractions.

2.5. Instrumental analysis

GC–MS analysis was carried out using a Hewlett-Packard HP gas chromatograph 5890 series II fitted with a 50 m×0.25 mm I.D. fused-silica column with a film thickness of 0.2 μm, coated with Carbowax 20M. The splitless/split injection port was heated to 200°C. Injection (2 μl) of the extract was done using an automatic sampler. The split vent was opened after 30 s. The carrier gas was helium 55 Norme Aga, and the pressure was 170 kPa, with a linear velocity of 40 cm s^{-1} at 40°C. The temperature program was 60°C (for 1 min), then increased at 4°C min^{-1} to 220°C and held at this temperature for a

further 20 min. The GC was coupled to a 5970 B mass-selective detector and a 5990 A MS chemstation (HP-UX). The interface was kept at 250°C and the ion source working in EI mode at 70 eV was held at 250°C. The quadrupole temperature was set at 250°C. The mass chromatograms were recorded by GC–MS operating in the selected ion monitoring mode to measure the ions $m/z=124$, 151 and 166 of MIBP and the ions $m/z=126$, 153 and 168 of [$^2H_2$]MIBP. Ions 124 and 126 were used for quantification and ions 151, 153, 166 and 168 were used as qualifiers. The calibration curve was established with standard mixtures containing defined amounts of labelled and unlabelled compound in different ratios, following the procedure recently published by Kotseridis et al. [14].

2.5.1. Reproducibility study

Five analysis of 100 ml of the same Merlot (1995 vintage) wine sample, spiked with 0.3 ng of MIBP [by adding 10 μl of a MIBP solution (30 ng ml^{-1}) in anhydrous alcohol], were carried out to study the reproducibility of the method described above.

2.6.1. Detection limit

A 100-ml volume of a Merlot wine (1995 vintage) and 100 ml of a Merlot grape juice (1996 vintage) were submitted to continuous extraction using 100 ml of dichloromethane to eliminate any trace of MIBP. Afterwards, the two matrices were spiked with 0.01, 0.1, 0.2 and 0.3 ng, respectively, of [$^2H_2$]MIBP [by adding 1, 10, 20 and 30 μl of a solution of [$^2H_2$]MIBP in anhydrous alcohol (10 ng ml^{-1}), respectively] and were submitted to the analysis described above. The limit of detection was taken to be the lowest amount giving a signal-to-noise ratio of three. For an injection of 2 μl of the wine or the grape juice extract, the detection limit was the average of five determinations of the lowest measurable peak area added with three times the standard deviation of this measurement [17].

3. Results and discussion

In this study, the deuterium-labelled internal standard used was 2-methoxy-3-([1,1-$^2H_2$]isobutyl)-pyrazine, labelled on the benzylic methylene of the isobutyl side chain. This was synthesised in a one-step reaction from the commercial unlabelled compound by simple imine–enamine chemistry, under acidic conditions. This was a relatively clean and high yielding reaction (86%) [14].

The relative abundances of three ions from the commercially available MIBP (124, 151, 166) and that of the [$^2H_2$]MIBP (126, 153, 168) were used in monitoring the endogenous MIBP in grapes and wines (Fig. 1).

The chosen isolation procedure was relatively simple, but non-selective. Amongst the 25 wines analysed during this assay and 48 samples (grapes/wines, maturation trials), quantification was not possible in only two cases (two Cabernet Sauvignon wines of 1995 vintage) due to coeluting material, assessed using the qualifier ions.

3.1. Extraction studies

The efficiencies of the extraction of MIBP from the same Merlot wine by four solvents were compared (Table 1).

Although, diethyl ether and dichloromethane were the solvents allowing the best recovery of the analyte, diethyl ether–hexane was chosen as its recovery yield was satisfactory and its affinity for compounds that can create interferences was lower than that of the first two solvents. In addition, the emulsion obtained with this solvent was less severe.

As regards the influence of pH value on the extraction recovery by diethyl ether–hexane, it was very low in wines but highly significant in grapes (Table 2). Thus, the pH of the grape juices was adjusted at 8.8 prior to analysis, but the wines were analysed directly at their natural pH.

Finally, the extraction by 3×5 ml diethyl ether–hexane was sufficient, as MIBP was undetectable by GC–MS in the fourth extraction.

3.1.1. Validation of the method

The square of the correlation coefficient of the regression line, obtained from the calibration data, was 0.999. The reproducibility was satisfactory as a coefficient of variation of 4.7% was obtained. Quantification was reliable down to 2 ng l^{-1}, with an estimated signal-to-noise ratio of 3:1 for a red 1995

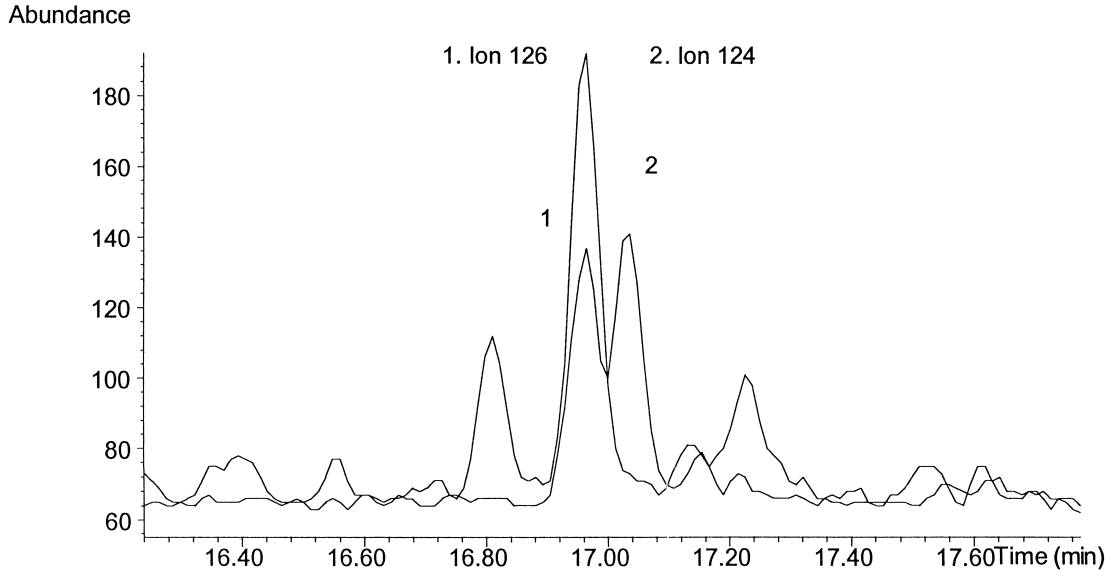

Fig. 1. Mass chromatogram of the extract of the Cabernet Sauvignon wine 1 (Table 4), from Medoc, vintage 1995.

Table 1
Recovery of MIBP from a Merlot wine, by different solvents

Type of solvent	Recovery (%)
Dichloromethane	94
Diethyl ether	95
Pentane–dichloromethane (1:2, v/v)	80
Diethyl ether–hexane (1:1, v/v)	90

Bordeaux wine (Merlot) and a Merlot grape sample (1996 vintage, Bordeaux).

The advantages of this method are the rapidity, simplicity and accuracy, as shown above. Less than 1 h was needed for the isolation, concentration and injection into the GC–MS system per sample, which is clearly shorter than the previous outlined methods, demanding two days per sample [9].

Table 2
Influence of pH on the recovery of MIBP from a Merlot wine and juice

pH value	Recovery yield (%)
pH of wine (3.8)	90
Wine adjusted to pH 8.8	92
pH of juice (3.5)	62
Juice adjusted to pH 8.8	89

3.2. Analysis of grape samples

The grapes were harvested in 1996 in various Bordeaux regions. The amounts of MIBP found in these samples were considerably lower than those previously reported in grapes of the same cultivars grown in other regions [11,15]. Of the three common varieties studied, the highest levels of MIBP were found in Cabernet Sauvignon grapes, followed by Cabernet franc and Merlot grapes. As shown in Table 3, technological maturity was achieved due to minimal fluctuations recorded in degrees Brix, from the first to the last date of harvest. The MIBP levels found in sound grapes were lower than the threshold value of MIBP in wines (10 ng l^{-1}), but were higher than its threshold level in water (0.5 ng l^{-1}). In the majority of the cases studied, the levels of MIBP remained consistent at near grape maturity. Only in the case of C. S. 2 Pauillac did we record a slight decrease in the MIBP levels.

When grapes became infected with the fungus *Botrytis cinerea*, MIBP values recorded were the highest. This observation could be explained by the infection of the corresponding grapes by the fungus *Botrytis cinerea* (V. Dupuch, personal communication) as the Brix values seen for Merlot 1 and

Table 3
°Brix (in grapes), total acidity (TA), pH, anthocyanins (mg l^{-1}) and MIBP levels in Merlot, Cabernet Sauvignon (C. S.) and Cabernet franc (C. f.) grapes (ng kg^{-1}) and in their corresponding wines (MIBP levels in ng l^{-1}) at different stages of maturity

	Grape samples					Wine samples				
	°Brix	TA (g l^{-1})	pH	Anthocyanins (mg l^{-1})	MIBP (ng kg^{-1})	Alcohol (%, v)	TA (g l^{-1})	pH	Anthocyanes (mg l^{-1})	MIBR (ng l^{-1})
Merlot 1 Margaux										
18 September 1996	22.7	3.3	3.4	261	3	12.8	3.5	3.4	587	9
23 September 1996	22.6	3.3	3.4	252	6	12.9	3.5	3.4	595	12
27 September 1996	22.5	2.9	3.4	262	2	12.8	3.3	3.4	640	7
30 september 1996	22.8	3.1	3.4	247	7	12.6	3.4	3.4	686	16
Merlot 2 Fronsac										
18 September 1996	23.6	3.1	3.4	207	5	13.7	3.7	3.4	648	3
23 September 1996	24.3	2.8	3.4	222	5	13.8	3.7	3.4	634	6
27 September 1996	24.1	2.8	3.5	238	5	13.1	3.6	3.4	655	3
30 September 1996	24.3	2.9	3.5	212	6	13.9	3.8	3.4	556	6
C.S. 1 Margaux										
26 September 1996	20.3	3.9	3.4	138	13	12.2	3.3	3.7	605	14
30 September 1996	21.7	4	3.5	194	7	12.1	3.5	3.7	584	9
03 October 1996	21.7	4.1	3.5	180	9	11.8	3.6	3.8	558	8
8 October 1996	22.1	4	3.5	184	20	11.9	3.4	3.8	485	19
C.S. 2 Pauillac										
26 September 1996	22.8	3.6	3.6	273	7	12.4	3.3	4.0	694	13
30 September 1996	22.4	3.4	3.7	340	6	12.7	3.6	3.9	730	12
03 October 1996	23.4	3.5	3.6	306	4	13.1	3.8	3.9	767	12
08 October 1996	23.4	3.5	3.6	306	4	12.9	3.6	3.9	827	8
C.f. 1 St. Emilon										
26 September 1996	22.6	3.9	3.4	111	5	12.6	3.1	3.9	484	3
30 September 1996	23.4	3.5	3.6	148	5	12.6	3.2	3.9	481	6
03 October 1996	23.2	3.5	3.5	116	7	13.1	3.3	4.0	530	6
07 October 1996	23.4	3.3	3.5	124	4	13.3	3.3	4.0	527	3
C.f. 2 St. Emilon M.										
26 September 1996	22.7	3.8	3.3	131	2	12.2	3.7	3.5	489	2
30 September 1996	22.3	4	3.4	133	7	12.5	3.8	3.4	484	6
03 October 1996	22.8	3.9	3.3	100	10	12.5	3.9	3.5	510	11
07 October 1996	22.8	3.5	3.4	128	4	12.6	3.8	3.5	519	7

Cabernet Sauvignon 1 (22.8 and 22.1, respectively) were high enough to facilitate this infection. Botrytis provoked shriveling of grapes, their skin was more fragile and the recovery of MIBP from the skins was easier.

Lacey et al. [11] analyzing grapes of Sauvignon blanc, sampled at ten-day intervals during ripening, concluded that the levels of 2-methoxy-3-alkylpyrazines decreased markedly in grapes as ripening increased, to reach a minimal level of MIBP that was close to 1 ng l^{-1}, at the last date of sampling. However, Allen et al. [15], reported an increase in MIBP levels in Sauvignon blanc grapes during ripening. In our study, the MIBP levels were always equal to or higher than 2 ng l^{-1} in the red grapes and wines analysed.

A linear trend was seen in all cases between MIBP levels in grapes and their corresponding wines. In particular, it was significant at the 5% level for Merlot 1 of Margaux ($R^2=0.97$; Fig. 2a) and Cabernet Sauvignon 1 of Margaux ($R^2=0.96$; Fig. 2c), but was not significant at the 5% level for Merlot 2 of

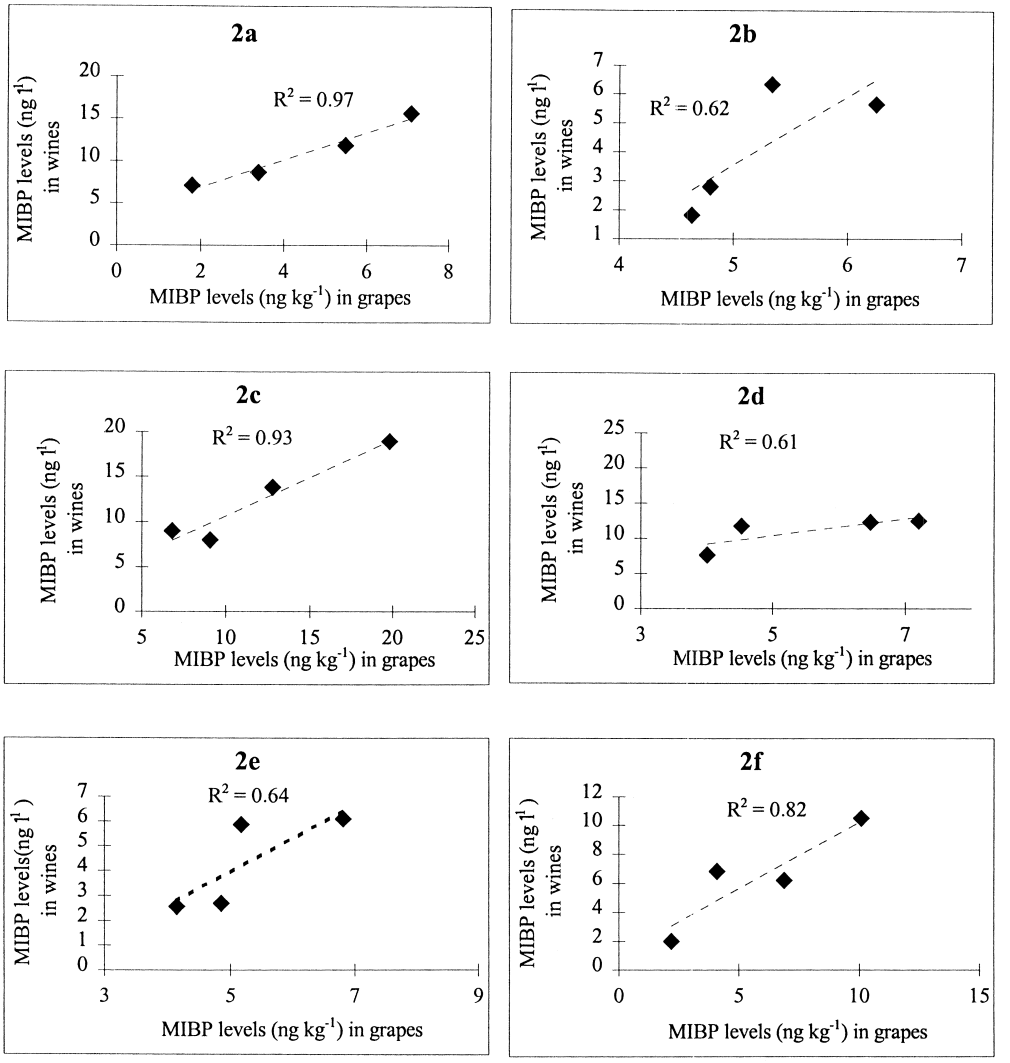

Fig. 2. Linear regression between MIBP levels in grapes and their corresponding wines (i) in Merlot [(a) Merlot 1 Margaux and (b) Merlot 2 Fronsac], (ii) in Cabernet Sauvignon [(c) Cabernet Sauvignon 1 Margaux and (d) Cabernet Sauvignon 2 Pauillac) and (iii) in Cabernet franc [(e) Cabernet franc 1 St. Emilon and (f) Cabernet franc 2 St. Emilon Montagne]

Fronsac (R^2=0.78; Fig. 2b) and Cabernet Sauvignon 2 of Pauillac (R^2=0.77; Fig. 2d).

In the case of Cabernet franc, a linear regression between MIBP levels in grapes and the corresponding wines was not significant at the 5% level in the St. Emilon samples (R^2=0.64; Fig. 2e), but it was nearly significant at the 5% level, in the St. Emilon Montagne samples (R^2=0.91; Fig. 2f). However, in all cases, MIBP levels in grapes and wines presented the same trend (Table 3).

3.3. Analysis of wine samples

MIBP levels for all of the wines analysed were found to be between 2 and 14 ng l^{-1}, which was in agreement with the amounts reported in red wines from the same regions [10]. All of the wines analysed came from wineries having the label ‘Grand Cru Classé’, i.e., they were the highest quality wines of that region, in which the odour descriptor bell pepper/vegetative, especially in the 1995 and 1996

vintages of great maturity (particularly for the 1995 vintage) was not frequently attributed. However, MIBP was identified in all of the wines, as shown in Table 4 and reached the levels of 12 and 13 ng l^{-1} in the Cabernet Sauvignon wines, i.e. higher than the threshold level of 10 ng l^{-1} in wine [6].

Another important point was the difference in the amounts of target compound between the 1995 and the 1996 Merlot and Cabernet Sauvignon wines. In 1996, the mean level in the Merlot wines (8 ng l^{-1}) amounted to 67% of the mean level in the Cabernet Sauvignon wines (12 ng l^{-1}). In 1995, a year known for the great maturity in Merlot grapes, the mean level in Merlot wines (4 ng l^{-1}) amounted to only 31% of the mean levels found in Cabernet Sauvignon wines (13 ng l^{-1}). Cabernet Franc cultivar, which is known to provide wines with intermediate analytical characteristics between the two previous cultivars, at least in the Bordeaux region, produced wines with intermediate MIBP levels in 1995, but in 1996, their MIBP mean level was even lower than that in Merlot wines. In 1996, the harvest of Merlot grapes was effected by rainfalls. Inversely, for the harvest of Cabernet franc and Cabernet Sauvignon grapes (two or three weeks later than for Merlot grapes), the climatic conditions were ideal. This could explain why half the mean levels of MIBP were found for Merlot wines of the 1995 vintage, in comparison to

Table 4
MIBP levels (ng l^{-1}) in wine samples from Bordeaux

Variety	Region	MIBP (ng l^{-1})		
		Sample 1	Sample 2	Mean value
	Wines from the 1996 vintage			
Merlot 1	Margaux	8	9	9
Merlot 2	Fronsac	6	7	6
Merlot 3	Graves	7	8	8
Merlot 4	Moulis	9	9	9
Merlot 5	Pomerol	12	12	12
Merlot 6	St. Emilon	7	7	7
Mean Merlot 1996				**8**
Cabernet Franc 1	St. Emilon	6	6	6
Cabernet Franc 2	St. Emilon	6	6	6
Mean CF 1996				**6**
Cabernet Sauvignon 1	Margaux	12	13	12
Cabernet Sauvignon 2	Pauillac	11	12	12
Cabernet Sauvignon 3	Graves	13	12	13
Cabernet Sauvignon 4	Moulis	10	10	10
Cabernet Sauvignon 5	Pauillac	11	12	11
Mean Cabernet Sauvignon 1996				**12**
	Wines from the 1995 vintage			
Merlot 1	Pomerol	3	2	3
Merlot 2	St. Emilon	5	4	4
Merlot 3	Graves	4	4	4
Merlot 4	Pomerol	4	5	5
Merlot 5	Moulis	4	4	4
Merlot 6	Pauillac	6	6	6
Mean Merlot 1995				**4**
Cabernet Franc 1	St. Emilon	5	5	5
Cabernet Franc 2	Pomerol	5	4	4
Mean cabernet Franc 1995				**5**
Cabernet Sauvignon 1	Medoc	14	14	14
Cabernet Sauvignon 2	Pauillac	12	11	11
Mean Cabernet Sauvignon 1995				**13**

the mean levels found for the wines of 1996 vintage, and the stability towards MIBP mean levels of Cabernet franc and Cabernet Sauvignon wines comparing the two vintages. Many authors have previously reported the influence of the vintage on MIBP levels in wines [6,10,12,15,18].

4. Conclusion

Quantitative analysis of MIBP in grapes and wines was achieved by an isotope dilution assay and applied to the three most frequently used varieties of the Bordeaux region. The levels found were in agreement with levels previously reported for wines from the same regions and the same varieties. The method was also used to study the evolution of MIBP levels in grapes and in the corresponding wines during ripening. In some cases, linear regression between MIBP levels in grapes and the corresponding wines was significant at the 5% level. Only in one case did the MIBP levels decrease with maturity.

The importance of the work illustrates the simple use of labelled MIBP in the detection of this highly potent compound, in trace levels, in grapes and wines. It also illustrates the ease of isolation of the natural compound in grapes and wines. Validation of the results, in more vintages and sampling dates, found for the correlation between the levels of MIBP in grapes and their corresponding wines could help analysts and oenologists make important decisions. Due to its high odour potency and its undesirable characteristics, the methods outlined for its isolation and for monitoring its levels, especially near grape maturation, in the vineyard provide an important tool for viticulturists/oenologists to predict the odour profiles in the finished wines.

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