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# Quantitative determination of β-ionone in red wines and grapes of Bordeaux using a stable isotope dilution assay

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**Abstract**

Quantitative analysis of β-ionone has been developed, using a stable isotope dilution assay. This was applied to red wines from different cultivars and regions. The Burgundy Pinot noir wines exhibited the highest levels of β-ionone. The variation in the levels of β-ionone in grape samples and in their corresponding wines, of Merlot, Cabernet Sauvignon and Cabernet franc from Bordeaux regions was monitored at four different stages towards the end of maturation : the levels of β-ionone were almost similar, exhibiting only a slight decrease during maturation. β-Ionone occurred in all the grapes and wines samples analysed at levels higher than, or close to its odour threshold which was determined in a model wine solution and was found to be of 90 ng $l^{-1}$. In a previously supplemented model wine solution with β-ionone (250 ng $l^{-1}$), the odour threshold was found to be of 980 ng $l^{-1}$ (total levels). 

*Keywords:* Wine; Food analysis; Grapes; Fruit; Ionones

## 1. Introduction

β-Ionone is an odourant compound used largely by the perfume and cosmetic industry for its typical violet odour [1]. Many authors have reported the occurrence of this compound in plants, fruits and flowers, which are rich in carotenoids; indeed β-ionone is a secondary metabolite generated by β-carotene, by either thermal degradation [2,3] or by photo-oxygenation [4].

It was first identified in wines of white grape cultivars in 1976 [5], but few papers, among the numerous ones dealing with the aroma of wines the last two decades, reported this powerful compound. It was identified in Californian Cabernet Sauvignon wines [6], in White Riesling juices [7], in Merlot noir clone wines [8] and was considered to be as one of the key compound of the aroma of Muscat de Frontignan [9] and of Grenache wines [10]. The average levels reported in the wines were lower than 450 ng $l^{-1}$ [8,10–12], much lower than the levels found by Etievant et al. [9]. A possible outcome for the low levels, could be the interactions with wine

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macromolecules and clarification enological products during the vinification process [13]. The low levels of this compound in wines, could explain why there are so few papers devoted to this powerful odourant of wine aroma.

The odour threshold in water of this compound was 30 ng $l^{-1}$ as re-determined recently [14]. These authors observed also that ca. 25% of the usually reliable judges seemed anosmic to even high levels of β-ionone. This could be a reason why high odour threshold were reported by Etievant et al. [9], 4.5 μg $l^{-1}$ in a base sweet white wine, and by Meilgaard [15] in beer.

Due to the great importance of β-ionone to wine aroma of many varieties and the large differences in the quantitative data reported in the literature, quantitative determination using a stable isotope dilution assay, seems to be indispensable. As earlier shown [16–18], a stable isotope dilution assay is an accurate method for the quantification of trace compounds. Thus, the aim of the following investigation was to develop such an assay, using an easily synthesised deuterium labelled β-ionone [12] and a convenient method for the isolation of the target compound, from grapes and wines of different varieties.

## 2. Experimental

### 2.1. Materials

Thirty seven pure Merlot, Cabernet Sauvignon, Cabernet Franc, Pinot Noir, Grenache and Xynomavro wines were analysed during this assay. The wines of the first three varieties were from Bordeaux regions (Margaux, Pauillac, Pomerol, Fronsac, Graves, Moulis and St. Emilion), the Grenache wines from Côtes du Rhône and Chateauneuf du Pape, the Pinot Noir from Burgundy, Alsace and Languedoc regions (France) and the Xynomavro wines from Naoussa and Amynteon regions (northern Greece). All the Bordeaux and Côtes du Rhône wines were prepared as reported elsewhere [8]. The Burgundy, Alsace, Languedoc and Greek wines were commercial and purchased from local market. The wines were between 1and 4 years old at the time of analysis.

Maturation trials: Six different vineyards were selected for this trial. The Merlot grapes were sampled from Margaux (gravely soil) and Fronsac (clayey-chalky soil), the Cabernet Sauvignon grapes from Margaux (gravely soil) and Pauillac (gravely-sandy soil), the Cabernet franc grapes from St. Emilion (clayey-chalky soil) and St. Emilion Montagne (sandy soil). Bunches of the 3 cultivars (70–80 kg per sampling) were harvested from vines at regular intervals (4 days) from a single row in each vineyard. Samples of 1000 berries were chosen for further analysis and stored at −20°C until analysis. The remainder grapes were used for producing the corresponding wines as reported elsewhere [8]. Four sampling and four corresponding vinifications were made for each cultivar.

β-ionone was purchased from Aldrich (Milwaukee, WI, USA). Details for the organic solvents used and the [$^2H_3$]β-ionone synthesis were described elsewhere [12].

### 2.2. Isolation of volatiles

Wine samples: 250 ml of a wine were placed in a closed flask, then spiked with 500 μl of [$^2H_3$]β-ionone (28.2 ng $ml^{-1}$, in ethanol) using a calibrated microliter syringe (SGE, 500 μl) and stirred for 10 min (equilibration time). It was then divided into two portions of 100 ml, placed into 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). Separation of the organic phase was done in a separatory funnel. The organic phases were blended, dried over $Na_2SO_4$, then filtered and concentrated under a nitrogen stream ($N_2$, 5.0 quality) down to 100 μl. The final concentration factor was 1000.

Grape samples: Berries samples (about 1 kg each) were allowed to reach 4°C overnight, then destemmed, crushed in a fruit-juicer for 2 min and centrifuged (9000 *g*, 15 min), while keeping the temperature at 4°C. Prior to analysis, the juices were filtered through glasswool, 250 ml of the sample was spiked with 500 μl of [$^2H_3$]β-ionone (28.2 ng $ml^{-1}$, in ethanol) and thereafter extracted as described above.

Each juice and wine sample was analysed in duplicate.

*2.2.1. Recovery from grapes and wines of added β-ionone*

A Merlot wine, spiked with 2 μg $l^{-1}$ of β-ionone, was submitted three times to liquid–1iquid extraction with solvents dichloromethane, diethyl ether, dichloromethane–pentane (1:2, v/v), and diethyl ether–hexane (1:1, v/v), as described above (2.2). After the extraction the organic phases of the same solvent were blended, spiked with 250 μl of [$^2H_3$]β-ionone (28.2 ng $ml^{-1}$, in ethanol) concentrated and injected in the GC–MS system. Furthermore, after the third extraction, the aqueous phase was extracted once more by 5 ml solvent, totalling four extractions. Each experiment was duplicated.

*2.3. Instrumental analysis*

Total sugars content, ethanol (%, v/v), total acidity and pH were determined on centrifuged juices and wines using standard procedures [19]. Determination of anthocyanins levels (mg $l^{-1}$) in grapes and in the corresponding wines was carried out by measurement of absorbance at 520 nm [20].

GC–MS analysis was carried out using a Hewlett-Packard HP gas chromatograph 5890 series II fitted with a 50 m BP 20 (Carbowax 20M) fused-silica column (0.25 mm I.D. and 0.2 μm film thickness, SGE). The splitless/split injection port was heated at 200°C. The 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 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 system was coupled to a 5970 B mass-selective detector and a 5990 A MS chemstation (HP-UX). The temperature of the ion source [working in the electron impact ionization (EI) mode at 70 eV], of the quadrupole and of the interface was set at 250°C. The selected ions of $m/z=177$, 192 were monitored for the determination of β-ionone and the selected ions of $m/z=180$ and 195 for [$^2H_3$]β-ionone. The ions of $m/z=177$, 180 were used for the quantitative determination and the ions of $m/z=192$, 195 as qualifiers. The calibration curve was established with standard mixtures containing defined amounts of labelled and unlabelled synthetic β-ionone in different ratios following the procedure described in [12]. Peak area ratios (peak area of the ion $m/z=177$/peak area of the ion $m/z=180$) were plotted against the concentration ratios (β-ionone/14.1 ng of [$^2H_3$]β-ionone) for the following (β-ionone concentrations: 7, 14, 20, 30 and 40 ng (three replicate analyses at each concentration). The resultant curve was linear [response ratio=(0.3615×concentration ratio)−0.1524; $R^2=0.999$].

*2.3.1. Reproducibility study and limit of reliable quantification*

Five analysis of 100 ml of the same Merlot wine (1995 vintage), containing 85 ng $l^{-1}$ β-ionone were carried out using the method described above to study its Reproducibility.

100 ml of a previously odour-stripped (by continuous extraction with 100 ml of dichloromethane) Merlot wine (1995 vintage) and Merlot grape juice (1995 vintage) were spiked with 0.184, 0.92, and 1.84 ng respectively of β-ionone [by adding 10, 50 and 100 μl of a β-ionone solution (18.4 ng $ml^{-1}$ in ethanol)] and were submitted to the isolation procedure as described above. The limit of reliable quantification was taken to be the lowest amount giving a signal-to-noise ratio of 3.

*2.4. Determination of the odour threshold of β-ionone in a model base wine*

Four concentrations of β-ionone, 80, 160, 640 and 960 ng $l^{-1}$ respectively were prepared in a model base wine (water–ethanol, 89:11, v/v; 1 l, tartaric acid 4 g and pH adjusted to 3.5 with $K_2CO_3$) from an initial solution of β-ionone (1.84 mg $ml^{-1}$ in ethanol). Another sensory experiment was set up, using the same model base wine to which 250 ng $l^{-1}$ of β-ionone were added previously. Four solutions were prepared as above, presenting total concentrations of β-ionone of 500, 750, 1250, and 2250 ng $l^{-1}$ respectively.

The olfactive perception threshold was determined using a triangle test. A 20 judge trained jury tasted the four series of three samples in covered glasses corresponding to AFNOR (Association Française des Normes) standards containing about 40 ml of liquid.

One sample contained the target compound dissolved in the model base wine, the other two samples were the model base wine. In another series of triangle tests, the presentation was reversed, two samples containing the target compound.

The determination of the odour threshold corresponded to the minimum concentration below which 50% of the judges failed to taste the difference from the control.

### *2.5. Statistical analysis*

Regression lines for the calibration curve and for the study of the relationship between β-ionone levels in grape and wine was performed with Excel package by Windows 7. The analysis of variance, was performed using same package.

## 3. Results and discussion

In this study the deuterium labelled compound, $[^2H_3]$β-ionone, used as internal standard was labelled at the C10 position in the side chain (megastigmane numbering system, [21]), synthesised in a one step synthesis from β-ionone, as reported previously [12].

Quantification was achieved by monitoring the $(M-15)^+$ fragment ion $m/z=177$ for the natural analogue and the corresponding $(M-15)^+$ fragment ion $m/z=180$ of $[^2H_3]$β-ionone (Fig. 1). These ions were the most abundant fragment ions in both of the compounds, which facilitate quantification of such a trace compounds. Moreover, the choice of these ions for the GC–MS quantification, was justified as interference with other potential co-eluting compounds was minimised and the signal-to-noise ratio was optimised, as shown in Fig. 1, presenting a GC–MS selected ion monitoring trace of a Merlot wine extract. At length, monitoring of two fragment ions of β-ionone $m/z=177$, 192 (and $m/z=180$, 195, respectively of $[^2H_3]$β-ionone) allowed to control the chromatographic purity of the peak of natural β-ionone in the analysed samples, by comparing their

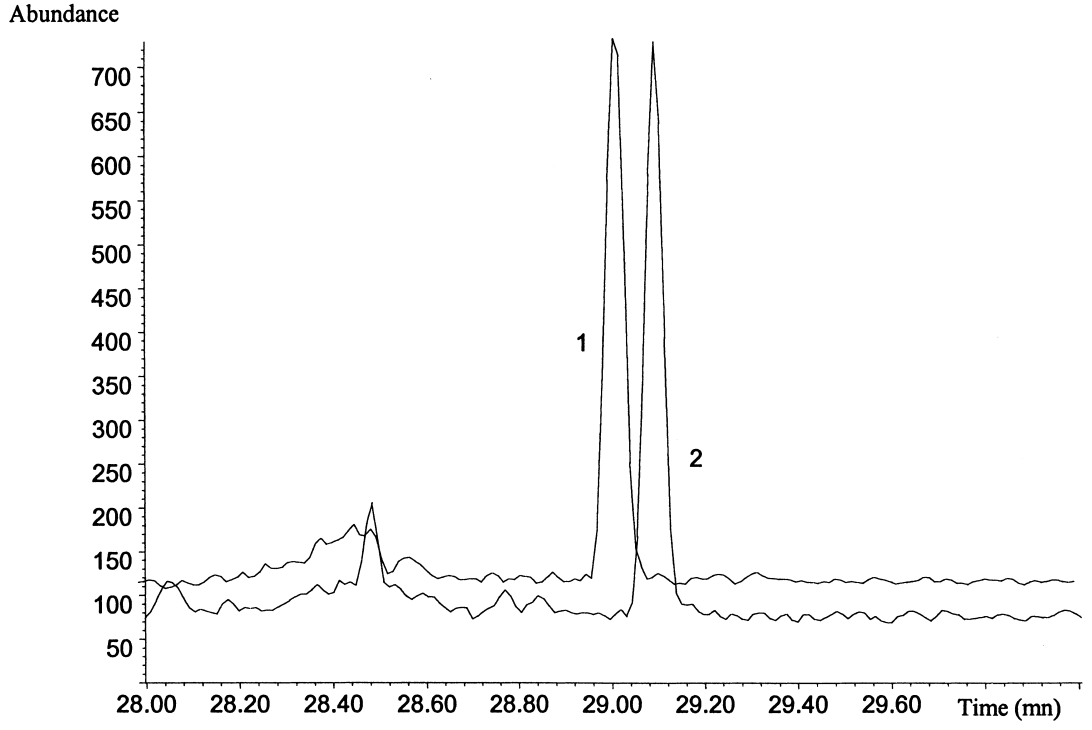


Fig. 1. Mass chromatogram of the diethyl ether–hexane (1:1, v/v) extract of a Cabernet Sauvignon wine, from Graves, vintage 1996. The level of β-ionone was 372 ng $l^{-1}$. Peaks: 1=$[^2H_3]$β-ionone, ion $m/z=180.00$; 2=β-ionone, ion $m/z=177.00$.

Table 1
Recovery of β-ionone from a Merlot wine by different solvents

| Type of solvent | Recovery (%) |
|---|---|
| Dichloromethane | 91 |
| Diethyl ether | 93 |
| Pentane–dichloromethane (1:2, v/v) | 73 |
| Diethyl ether–hexane (1:1, v/v) | 86 |

relative abudances. In none of the 85 analysed samples any interference, with the natural or the deuterium labelled compound, was detected.

### 3.1. Extraction studies

Four solvent for the recoveries of β-ionone from the same Merlot wine using the isolation procedure described above were compared (Table 1).

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

Finally the extraction by 3×5 ml diethyl ether–hexane was sufficient, as in a fourth extraction no β-ionone was detected by GC-MS.

#### 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% was obtained. Quantification was reliable down to 9 ng $l^{-1}$ with an estimated signal to noise ratio of 3:1 for an odour stripped red wine and grape sample (Merlot 1995, from Bordeaux). Furthermore, the concentration at which the signal-to-noise ratio became 3, was estimated to be 12 ng $l^{-1}$, in the sample presenting the lowest β-ionone level (Xynomavro 2, Table 4).

### 3.2. Odor threshold of β-ionone in a model base wine

The odour thresholds of β-ionone in wine and beer, 4500 and 1600 ng $l^{-1}$, respectively [9,15], were much higher than those reported in water (30 ng $l^{-1}$ [14]). Therefore we determined its threshold in a model base wine. The value found, 90 ng $l^{-1}$ (Table 2), was close to that reported in water. The high levels determined in wine and beer could result from the fact that the authors of these experiments were not aware of the occurrence of β-ionone trace levels in the wine and beer used as the controls, due to the low analytical performances at that time (1975 and 1983). Thus, these values could be in fact difference thresholds, which are usually higher than their corresponding odour thresholds. To confirm this hypothesis, the same model wine used for the determination of the odour threshold was supplemented with 250 ng $l^{-1}$ i.e. about the mean value of the β-ionone levels found in the wines analysed during this study. The odour threshold found in these conditions was 980 ng $l^{-1}$ i.e. 11 times higher than in a free β-ionone model wine. Therefore, the odour threshold of β-ionone in wine will be approximated

Table 2
Odour threshold of β-ionone in a model solution and in an added with β-ionone (250 ng model solution

| | Number of total answers | Number of correct answers | Concentrations of β-ionone (ng) | $\log_{10}$ of conc. | O.t.[a] at 50 % |
|---|---|---|---|---|---|
| Model base wine | 20 | 8 | 80 | 1.90 | |
| | 20 | 16 | 160 | 2.20 | 90 ng $l^{-1}$ |
| | 20 | 18 | 640 | 2.81 | |
| | 20 | 20 | 960 | 2.98 | |
| Model base wine | 20 | 2 | 500[b] | 2.70[b] | |
| containing | 20 | 8 | 750[b] | 2.88[b] | 980 ng $l^{-1}$ |
| β-ionone (250 ng $l^{-1}$) | 20 | 12 | 1250[b] | 3.10[b] | |
| | 20 | 20 | 2250[b] | 3.35[b] | |

[a] O.t.: Odour threshold.
[b] Total β-ionone concentration.

in this study by that determined in the model base wine, i.e. 90 ng $l^{-1}$

### 3.3. Variation of β-ionone levels during maturation

Twenty-four grape samples and their corresponding wines were analysed during this study. The grapes were sampled between 18 September and 7 October 1996, every 4 days from each vineyard (Table 3). The levels of β-ionone found were between 81 to 337 ng $l^{-1}$ (Table 3). The wines exhibited generally levels higher than, or equal to those found in grapes. The only significant difference was found in the case of Merlot 1 Margaux [$F(1, 6)=112.7$, $p=4.1\times10^{-5}$], in which the levels in wine were significantly higher than in grapes. A slight decrease was observed in the grapes during ripening, which could be explained by a decrease of

Table 3
pH and levels of sugar (°Brix ; grapes), alcohol (wines), total acidity (TA), anthocyanes and β-ionone in grapes and wines during ripening

| Samples | Grapes | | | | | | | Wines | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | °Brix | Ta ($g\,l^{-1}$) | pH | Anthocyanes ($mg\,l^{-1}$) | β-Ionone ($ng\,hg^{-1}$) | | | Alcohol (%, v/v) | TA ($g\,l^{-1}$) | pH | Anthocyanes ($mg\,l^{-1}$) | β-Ionone ($ng\,l^{-1}$) | | |
| | | | | | Sample 1 | Sample 2 | Mean value | | | | | Sample 1 | Sample 2 | Mean value |
| Merlot 1 Margaux | | | | | | | | | | | | | | |
| 18 Sept. 1996 | 22.7 | 3.3 | 3.41 | 261 | 143 | 143 | 143 | 12.8 | 3.5 | 3.45 | 587 | 304 | 288 | 296 |
| 23 Sept. 1996 | 22.6 | 3.3 | 3.4 | 252 | 147 | 149 | 148 | 12.9 | 3.5 | 3.46 | 595 | 269 | 279 | 274 |
| 27 Sept. 1996 | 22.5 | 2.9 | 3.49 | 262 | 139 | 133 | 136 | 12.8 | 3.3 | 3.47 | 640 | 287 | 282 | 285 |
| 30 Sept. 1996 | 22.8 | 3.1 | 3.45 | 247 | 115 | 120 | 118 | 12.6 | 3.4 | 3.45 | 686 | 341 | 333 | 337 |
| Merlot 2 Fronsac | | | | | | | | | | | | | | |
| 18 Sept. 1996 | 23.6 | 3.1 | 3.45 | 207 | 110 | 110 | 110 | 13.7 | 3.7 | 3.4 | 648 | 184 | 182 | 183 |
| 23 Sept. 1996 | 24.3 | 2.8 | 3.49 | 222 | 116 | 118 | 117 | 13.8 | 3.7 | 3.45 | 634 | 187 | 192 | 190 |
| 27 Sept. 1996 | 24.1 | 2.8 | 3.53 | 238 | 93 | 93 | 93 | 13.1 | 3.6 | 3.45 | 655 | 118 | 122 | 120 |
| 30 Sept. 1996 | 24.3 | 2.9 | 3.59 | 212 | 80 | 82 | 81 | 13.9 | 3.8 | 3.45 | 556 | 90 | 94 | 92 |
| C.S.[a] 1 Margaux | | | | | | | | | | | | | | |
| 26 Sept. 1996 | 20.3 | 3.9 | 3.48 | 138 | 294 | 304 | 299 | 12.2 | 3.3 | 3.75 | 605 | 289 | 297 | 293 |
| 30 Sept. 1996 | 21.7 | 4 | 3.57 | 194 | 267 | 267 | 267 | 12.1 | 3.5 | 3.7 | 583 | 234 | 240 | 237 |
| 3 Oct. 1996 | 21.7 | 4.1 | 3.52 | 180 | 203 | 210 | 207 | 11.8 | 3.6 | 3.8 | 558 | 208 | 200 | 204 |
| 8 Oct. 1986 | 22.1 | 4 | 3.54 | 184 | 141 | 139 | 140 | 11.9 | 3.4 | 3.85 | 485 | 183 | 181 | 182 |
| C.S. 2 Pauillac | | | | | | | | | | | | | | |
| 26 Sept. 1996 | 22.8 | 3.6 | 3.6 | 273 | 220 | 212 | 216 | 12.4 | 3.3 | 4.0 | 694 | 289 | 293 | 291 |
| 30 Sept. 1996 | 22.4 | 3.4 | 3.72 | 340 | 192 | 194 | 193 | 12.7 | 3.6 | 3.95 | 730 | 196 | 190 | 193 |
| 3 Oct. 1996 | 23.2 | 3.8 | 3.63 | 250 | 221 | 215 | 218 | 13.1 | 3.8 | 3.9 | 767 | 173 | 179 | 176 |
| 8 Oct. 1996 | 23.4 | 3.5 | 3.68 | 306 | 216 | 214 | 215 | 12.9 | 3.6 | 3.95 | 827 | 166 | 166 | 166 |
| C.f.[b] 1 St. Emilon | | | | | | | | | | | | | | |
| 26 Sept. 1996 | 22.6 | 3.9 | 3.44 | 111 | 163 | 163 | 163 | 12.6 | 3.1 | 3.9 | 484 | 266 | 277 | 272 |
| 30 Sept. 1996 | 23.4 | 3.5 | 3.6 | 148 | 189 | 190 | 190 | 12.6 | 3.2 | 3.9 | 481 | 178 | 170 | 174 |
| 3 Oct. 1996 | 23.2 | 3.5 | 3.58 | 116 | 155 | 161 | 158 | 13.1 | 3.3 | 4.0 | 530 | 166 | 160 | 163 |
| 7 Oct. 1996 | 23.4 | 3.3 | 3.57 | 124 | 160 | 153 | 157 | 13.3 | 3.3 | 4.0 | 527 | 186 | 194 | 190 |
| C.f. 2 St. Emilon M. | | | | | | | | | | | | | | |
| 26 Sept. 1996 | 22.7 | 3.8 | 3.36 | 131 | 194 | 188 | 191 | 12.2 | 3.7 | 3.5 | 489 | 254 | 260 | 257 |
| 30 Sept. 1996 | 22.3 | 4 | 3.42 | 133 | 184 | 190 | 187 | 12.5 | 3.8 | 3.45 | 484 | 194 | 202 | 198 |
| 3 Oct. 1996 | 22.8 | 3.9 | 3.49 | 100 | 156 | 144 | 150 | 12.5 | 3.9 | 3.5 | 510 | 193 | 193 | 193 |
| 7 Oct. 1996 | 22.8 | 3.5 | 3.41 | 128 | 224 | 222 | 223 | 12.6 | 3.8 | 3.55 | 519 | 130 | 130 | 130 |

[a] C.S.: Cabernet Sauvignon.
[b] C.f.: Cabernet franc.

the levels of β-carotene, as reported previously [22]. However in the case of Cabernet franc 1 of St. Emilion the levels at the last date of sampling were as low as those at the first date and in the case of Cabernet franc 2 of St. Emilion the levels of the last sampling were the highest.

The relationship between the levels of β-ionone found in grapes and in their corresponding wines was tested using linear regression. In only one case (Merlot 2 Fronsac, Fig. 2b) the linear regression was significant at the 1% level [$F(1, 2)=108.5$, $p=0.01$, linear regression slope=2.9], but in the case of Merlot 1 Margaux (Fig. 2a) and of Cabernet Sauvignon 1 Margaux (Fig. 2c) the relationship was only significant at the 7% [$F(1, 2)=12.2$, $p=0.07$, linear regression slope=−1.9] and 6.3% [$F(1, 2)=14.4$, $p=0.063$, linear regression slope=0.65] levels respectively. The fact that β-ionone could be produced during the crushing of grapes by oxidation of β-carotene could explain the weak relationships between the levels of β-ionone found in grapes and in their corresponding wines. Furthermore, the interac-

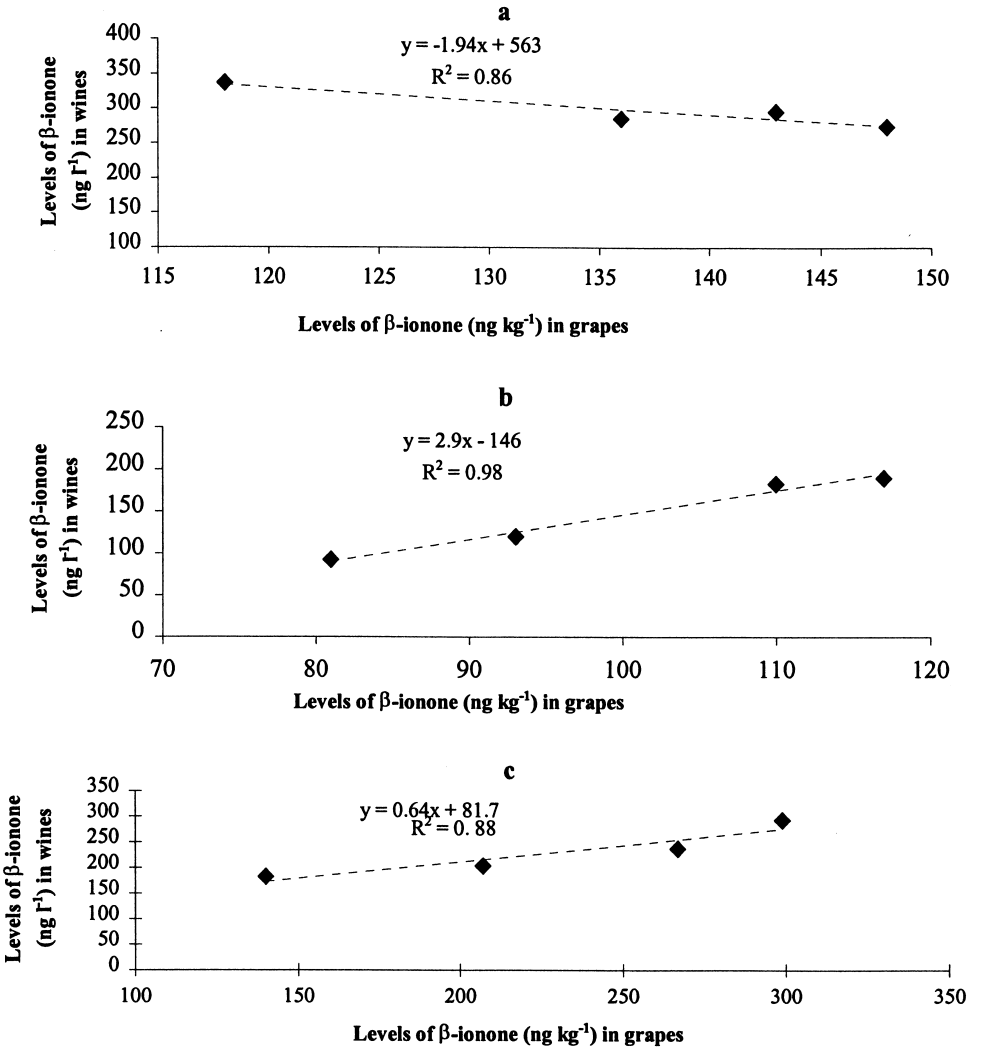


Fig. 2. Linear regression between the levels of β-ionone found in grapes (ng $kg^{-1}$) and in their corresponding wines (ng $l^{-1}$). (a) Merlot 1 Margaux, (b) Merlot 2 Fronsac and (c) Cabernet Sauvignon 1 Margaux.

tions with wine macromolecules and clarification enological products [13], during the vinification process, could influence the β-ionone levels.

### 3.3.1. Analysis of wine samples

Twenty wines of Merlot, of Cabernet franc and of Cabernet Sauvignon from different Bordeaux regions and from two vintages 1995 and 1996 were analysed during this study (Table 4). Cabernet Sauvignon wines exhibited levels significantly higher than the Merlot wines levels [$F(1, 15)=8.63$, $p=0.01$], but not significantly higher than the Cabernet franc wines levels. The levels in these last two varieties showed no significant difference. No significant difference was observed between the mean levels in the Merlot wines of 1995 (143 ng $l^{-1}$) and of 1996 (162 ng $l^{-1}$) [$F(1, 10)=0.47$, $p=0.51$], as well as between those of the 1995 and 1996 Cabernet Sauvignon wines [$F(1, 3)=0.28$, $p=0.63$], 217 ng $l^{-1}$ and 256 ng $l^{-1}$ respectively. As regards the wines from the other varieties, the levels found in Grenache wines (258 ng $l^{-1}$) were not significantly different from those found in Cabernet Sauvignon wines, contrary to the wines of Xynomavro, which contained the lowest levels of the 37 wines analysed and the Pinot noir wines of Burgundy which contained the highest levels (mean level higher than 1000 ng $l^{-1}$). As these two Burgundy Pinot noir wines were commercial, their high levels could be due to barrel ageing, as the occurrence of β-ionone

Table 4
Levels of β-ionone (ng $l^{-1}$) found in wines from different varieties, regions and vintages

| Bordeaux wines | | | | | | Wines from different regions | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Variety | Region | Vintage | Sampe 1 | Sample 2 | β-Ionone (ng $l^{-1}$) mean value | Variety | Region | Vintage | Sample 1 | Sample 2 | β-Ionone (ng $l^{-1}$) mean value |
| Merlot 1 | Margaux | 1996 | 154 | 158 | 156 | Grenache 1 | Chateuneuf | 1995 | 175 | 179 | 177 |
| Merlot 2 | Fronsac | 1996 | 90 | 97 | 94 | Grenache 2 | Chateuneuf | 1995 | 200 | 192 | 196 |
| Merlot 3 | Graves | 1996 | 183 | 175 | 179 | Grenache 3 | Chateuneuf | 1995 | 201 | 208 | 205 |
| Merlot 4 | Moulis | 1996 | 135 | 142 | 139 | Grenache 4 | Côtes du Rhône | 1995 | 327 | 329 | 328 |
| Merlot 5 | Pommerol | 1996 | 150 | 144 | 147 | Grenache 5 | Côtes du Rhône | 1995 | 385 | 385 | 385 |
| Merlot 6 | St. Emillon | 1996 | 254 | 260 | 257 | | | | | | |
| Mean Merlot | | | | | | Mean Grenache | | | | | |
| 1996 | | | | | 162 | 1995 | | | | | 258 |
| Merlot 1 | Pommerol | 1995 | 118 | 109 | 114 | Xynomavro 1 | Naoussa | 1994 | 82 | 91 | 87 |
| Merlot 2 | St Emillon | 1995 | 73 | 77 | 75 | Xynomavro 2 | Naoussa | 1994 | 61 | 63 | 62 |
| Merlot 3 | Graves | 1995 | 170 | 172 | 171 | Xynomavro 3 | Amynteon | 1995 | 135 | 135 | 135 |
| Merlot 4 | Pommerol | 1993 | 187 | 193 | 190 | Xynomavro 4 | Amynteon | 1995 | 144 | 141 | 142 |
| Merlot 5 | Moulis | 1995 | 140 | 130 | 135 | Xynomavro 5 | Naoussa | 1995 | 120 | 126 | 123 |
| Merlot 6 | Pauilluc | 1995 | 168 | 173 | 171 | Xynomavro 6 | Naoussa | 1995 | 110 | 120 | 115 |
| Mean Merlot | | | | | | Mean Xynomavro | | | | | 111 |
| 1995 | | | | | 143 | | | | | | |
| C. Franc l | St Emillon | 1995 | 150 | 154 | 152 | C. Sauvignon 1 | Rioja | 1996 | 101 | 105 | 103 |
| C. Franc 2 | St Emillon | 1995 | 155 | 151 | 153 | | | | | | |
| C. Franc 3 | Pommerol | 1995 | 149 | 144 | 147 | | | | | | |
| Mean C. Franc | | | | | | | | | | | |
| 1995 | | | | | 151 | | | | | | |
| C. Sauvignon 1 | Pauillac | 1996 | 196 | 196 | 196 | Pinot Noir 1 | Santenay/Burgundy | 1996 | 790 | 790 | 790 |
| C. Sauvignon 2 | Graves | 1996 | 372 | 372 | 372 | Pinot Noir 2 | Pommard/Burgundy | 1995 | 1476 | 1474 | 1475 |
| C. Sauvignon 3 | Moulis | 1996 | 198 | 204 | 201 | Pinot Noir 3 | Languedoc | 1996 | 197 | 193 | 195 |
| Mean C. Sauvignon | | | | | | Pinot Noir 4 | Languedoc | 1994 | 152 | 152 | 152 |
| 1996 | | | | | 256 | Pinot Noir 5 | Alsace | 1993 | 82 | 90 | 86 |
| C. Sauvignon 1 | Medoc | 1995 | 232 | 230 | 231 | | | | | | |
| C. Sauvignon 2 | Pauillac | 1995 | 199 | 205 | 202 | | | | | | |
| Mean C. Sauvignon | | | | | | | | | | | |
| 1995 | | | | | 217 | Mean Pinot Noir | | | | | 540 |

[23] and of β-carotene [24] were reported in oakwood sources. However no toasty character were detected in these Pinot noir wines by tasting. The levels found in the other three Pinot noir commercial wines from Languedoc and Alsace were significantly lower than those found in the Burgundy wines [$F(1,3)=14.6$, $p=0.03$], although the Languedoc samples were aged in barrel as mentioned on the bottle. This variation of the β-ionone levels could be due to the origin of Pinot noir, to different winemaking processes, or the barrels used. Indeed, it was reported [24] that the β-carotene levels in oak barrels depended on species, origin, toasting and age.

Finally it must be noted that the levels found in all the wines analysed, excepted in one Merlot and in one Xynomavro sample, were higher than the odour threshold of β-ionone determined in a model base wine (see above).

## 4. Conclusion

The β-ionone levels found in all the wines analysed relatively to its odour threshold showed the importance of this trace compound in wine aroma. The levels of β-ionone in grapes and in the corresponding wines decreased slightly during ripening, but they were always higher than its odour threshold.

The weak relationships observed between the levels of β-ionone in grapes and in their corresponding wines could be due to the sample handling under aerobiosis conditions and/or to the interactions of this compound with wine macromolecules. In future, monitoring this important odourant at technological maturity under inert atmosphere (oxygen free), oenologist and food analyst can gather insight to the β-ionone levels in their grapes and wines.

Finally, experimental Pinot noir wines from Burgundy and from other regions should be analysed to explain the difference observed on the commercial Pinot noir wines.

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