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A Survey of Seasonal Temperatures and Vineyard Altitude Influences on 2-Methoxy-3-isobutylpyrazine, C_{13}-Norisoprenoids, and the Sensory Profile of Brazilian Cabernet Sauvignon Wines

LEILA DENISE FALCÃO,[†] GILLES DE REVEL,[‡] MARIE CLAIRE PERELLO,[‡] ANASTASIA MOUTSIOU,[‡] MAURO CELSO ZANUS,[§] AND MARILDE T. BORDIGNON-LUIZ*[,†]

Departamento de Ciência e Tecnologia de Alimentos CAL/CCA, Universidade Federal de Santa Catarina, Rod. Admar Gonzaga, 1346, Itacorubi, CEP 88034-001, Florianópolis, SC, Brazil, Faculté d'Œnologie, ISVV, UMR 1219 INRA, Université Victor Segalen Bordeaux 2, 351 Cours de la Libération, F-33405 Talence, France, and Embrapa Uva e Vinho, Rua Livramento, 515, Caixa Postal 130, CEP 95700-000, Bento Gonçalves, RS, Brazil

This report has investigated the seasonal temperatures influences (winter and summer) of five vineyards at different altitudes on the concentrations of 2-methoxy-3-isobutylpyrazine (MIBP), α- and β-ionone, and β-damascenone in 2004 or 2005 vintages of Cabernet Sauvignon wines from Santa Catarina State, Brazil. Sensorial analyses were also carried out on the wine samples and compared to altitude and climate. Significant regression was observed between MIBP concentrations and the vineyard's altitude. No significant relation was observed between α- and β-ionone and β-damascenone with the vineyard's altitude. Principal component analysis positively correlated wines from higher altitudes with a "bell pepper" aroma. Conversely, the wines made with grapes from lower altitudes were correlated with "red fruits" and "jam" aromas. An important relation between the bell pepper aroma and the lower winter temperature was observed. A strong negative correlation was also observed between seasonal temperatures and vineyard altitude as well as between MIBP content and seasonal temperature of growing grapevines.

KEYWORDS: Cabernet Sauvignon wine; MIBP; α- and β-ionones; β-damascenone; seasonal climate

INTRODUCTION

Extensive research has been undertaken to identify key aroma components present in wine. Some of the most important include 2-methoxy-3-isobutylpyrazine (MIBP) (*1*−*8*), α- and β-ionone, and β-damascenone (*6*, *9*−*15*). The aroma described as vegetal, herbaceous, or "bell pepper" in wines of grape varieties such as Cabernet Sauvignon, Sauvignon blanc, Cabernet Franc, and Merlot has been attributed to the presence of methoxypyrazine compounds (*2*, *8*). In 1975, MIBP was reported in Cabernet Sauvignon grapes (*4*) and its sensory detection threshold in red wine has been reported as 10−16 ng/L (*6*, *16*) with 15 ng/L on average (*16*). In contrast to the intensely flavored wines of Muscat and floral grapes, varieties such as Cabernet Sauvignon contain glycosidic precursors of C_{13} norisoprenoid components, which are important sources of grape-derived flavors in wines (*15*). Norisoprenoids commonly found as secondary metabolites originating from the enzymatic oxidation of carotenoids and α- and β-ionone have detection thresholds in wines, respectively, of 90 (*11*) and 400 ng/L (*17*). α-Ionone has multiple descriptors, including violets, floral, and fruity, whereas β-ionone has been described as dry fruit and raspberry-like. β-Damascenone is particularly interesting, since several other precursors have been found that are believed to arise through the degradation of allenic carotenoids, such as neoxanthin, and the subsequent glycosylation of the derived alcohols. This norisoprenoid ketone is principally generated from hydrolyzable precursors (*12*). It exhibits a complex aroma, which, depending on its concentration, is reminiscent of tropical flowers with fruity and berry undertones (*13*). Its sensory detection threshold is 50 ng/L in hydroalcoholic solution (*10*), which indicates that it may be important to wine flavor.

There are many factors including the effect of the terroir (soil, terrain, climate, etc.) capable of influencing the vintage, and the role exerted by each individual factor is still not clearly established. Altitude could exert an important effect on grape maturation and the composition of wine that is strictly related to the local climate. The effects of vineyard altitude have been published on the levels of proanthocyanidins and anthocyanins in wines (*18*, *19*) and of monoterpenes and norisoprenoids in grapes (*14*).

* To whom correspondence should be addressed. Tel: +55(0)48 3721 5376. Fax: +55(0)48 3331 9943. E-mail: bordign@cca.ufsc.br; leiladfalcao@yahoo.com.br.
[†] Universidade Federal de Santa Catarina.
[‡] Université Victor Segalen Bordeaux 2.
[§] Embrapa Uva e Vinho.

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Table 1. Characteristics of the Vineyards from Different Altitudes Used in the Study[a]

wine samples	localization (latitude and longitude)	altitude (m)	conduction system	rows × vines spaces (m^2)	rootstock	clone
774	27° 0′ 14″ and 51° 9′ 0″	774	V system	3.0 × 1.50	Paulsen 1103*	R-5
960	27° 51′ 80″ and 49° 35′ 43″	960	V system	3.0 × 1.20	Paulsen 1103	685
1160	28° 19′ 0″ and 49° 34′ 51″	1160	V system	3.2 × 1.30	Paulsen 1103	R-5
1350	26° 43′ 30″ and 49° 55′ 60″	1350	vertical trellis system	3.0 × 1.50	Paulsen 1103	R-5
1415	28° 16′ 41″ and 49° 55′ 96″	1415	V system	3.5 × 1.50	Paulsen 1103	**

[a] *, *V. berlandieri* × *V. ruprestris*; **, unknown.

The measurement of wine flavor is important to characterize the wine in relation to the terroir. However, MIBP, α- and *β*-ionone, and *β*-damascenone occur in grape juices and young wines at low concentrations as compared to other volatiles, making quantitative analyses difficult.

In Santa Catarina State, southern Brazil, the altitudes of vineyards can differ by as much as 700 m and climatic conditions vary as a consequence. The Cabernet Sauvignon wines used in this study are produced by two main viticulturist regions of Santa Catarina State, in the Planalto Sul Catarinense and in the Vale do Rio do Peixe. In the first region is the town of São Joaquim, which is known in Brazil as the coldest place in the country.

In this study, a simple gas chromatography−mass spectrometry (GC-MS) method was used to quantify MIBP, α- and *β*-ionone, and *β*-damascenone in Cabernet Sauvignon wines from five vineyards at different altitudes in Santa Catarina State, Brazil, using the 2004 and 2005 vintages. Seasonal temperatures from vineyards at different altitudes were investigated. Sensory analysis was carried out on the wines, and principal component analysis (PCA) was applied. Chemical data together with the vineyard's altitudes and seasonal temperatures were subjected to correlation analysis.

MATERIALS AND METHODS

Samples. Wines from the 2004 and 2005 vintages of the Cabernet Sauvignon variety taken from five sites of Santa Catarina State (SC) at different altitudes were analyzed as follows: codes 1415 and 1160 correspond to São Joaquim, at 1415 and 1160 m asl; code 1350 corresponds to Água Doce, town at 1350 m asl; code 960 corresponds to Bom Retiro, a town at 960 m asl; and code 774 corresponds to Videira, at 774 m asl. The samples consisted of a total amount of nine wines, five from 2004 (-04) and four from 2005 (-05). Experimental plots of Cabernet Sauvignon were delimited in young commercial vineyards and used to make the wines, although some difficulties were encountered with different conduction systems, vine age, planting density, and clone type. Vine ages were between 4 and 5 years and, in yield, from 8 to 10 t/ha. The characteristics of each vineyard are presented in **Table 1**.

Two replicated wine fermentations were prepared for each sample. The wines were produced under the same conditions of microvinification at EPAGRI (Empresa de Pesquisa e Extensão Agropecuária de Santa Catarina), in Videira, SC, Brazil: The grapes were separated from the stalks, crushed, and maintained in a 20 L capacity stainless steel vat. The maceration period was 10 days, with two daily reconstitutions at 22 °C. The must was separated from the solid parts and transferred to 13 L capacity stainless steel vats. Prior to initiating alcohol fermentation, a commercial sulfiting agent (20 g/100 kg of must, corresponding to 10 mg/L of free SO_2) (Noxitan, Pascal Biotech, Paris), *Saccharomyces cerevisae* strain (20 g/100 kg) (Fermol Rouge, Pascal Biotech), and commercial enzymes with pectinolytic activity (2−4 g/hL) (Pectinex SPL/Ultra, Pascal Biotech) were added to the musts. Malic acid consumption by lactic bacteria occurred spontaneously within 20−25 days. Once the alcohol fermentation had finished, the wines were chilled to −4 °C for 10 days, Noxitan (35 mg/L of free SO_2, on average) was added, and then, they were bottled. All of the samples were between 14 and 20 months old at the time of analysis. The 2004 and 2005 wine samples were stored at 5 °C prior to analysis and were analyzed at Bordeaux University.

Climate Data. Climate data used in this study were from meteorological stations situated either within or in close proximity to the vineyards, except for the site at 1160 m (1160 wine), for which data from a station located 4 km from the vineyard was used. The data consisted of daily observations of maximum, minimum, and mean temperatures, thermal amplitude, and rainfall.

Classical Wine Analysis. Malic acid was quantified using an enzymatic kit (Boehringer, Mannheim, Germany/R-Biopharm), and the results were expressed in g/L. Total acidity, volatile acidity (VA), ethanol, and pH were carried out according to protocols established by the OIV (*20*).

Sensory Testing. A generic quantitative descriptive analysis (QDA) method was used according to AFNOR (NF V 09-016, 1995) (*21*). The 2004 wines were stored at Bordeaux University at 5 °C and were brought into the sensory room at least 3 h before testing to equilibrate with the testing environment. Fifteen minutes before the start of the tasting session, 40 mL portions of the wines were poured into clear, tulip-shaped 250 mL glasses that were coded with three-digit random numbers. The tasting sessions were carried out in an acclimatized sensory room (18 °C), composed of individual cabins under "sodium" lighting. The order of sample presentation was completely random. For the 2004 vintage samples, nine previously defined descriptors, four by taste and five aromas, were evaluated on a line scale of 0−9 (0 = not present and 9 = extremely intense). A qualified panel formed of 14 persons, all enologists of the second year of the Diplôme National d'Oenologue, Faculté d'Oenologie at Bordeaux University, eight males and six females, aged 22 to 47, evaluated the 2004 vintage wines on January 12, 2006. The panel was experienced in QDA on the Cabernet Sauvignon wines. Thus, the first bottle of each wine was opened for the panel to generate the descriptors in the sensorial session week. The second bottle of each wine was opened for the sensory testing. The data were collected using FIZZ software (Biosystems, Couternon, France). For the 2005 vintage wines, the same conditions mentioned above were followed, except that 10 previously defined descriptors were determined, four by taste and six aromas, and dark, tulip-shaped 250 mL glasses were used. A qualified panel formed by 13 judges, enologists and expert judges from the EMBRAPA (Empresa Brasileira de Pesquisa e Extensão Agropecuária, Bento Gonçalves, Brazil), ten males and three females, aged 27 to 64, was invited to evaluate the wines on July 7, 2006. The judges were selected based on experience in QDA and their expectation in participating in the research. The tasting sessions lasted approximately 15−20 min. The same facilitator guided both sensory testing sessions.

Sensory Panel Training. For 2005 vintage, panelists attended three 1 h sessions in a week. The panelists were experienced in sensory descriptive analysis and easily generated terms to describe the wines of their interest on the first session. Panelists first listed descriptors for the wines individually, then discussed the terms as a group, and finally ranked the wines for the attributes. If there were disagreements among the panelists, the group discussed their differences and came to a consensus. At the second session, the panelists were given a list of all of the attributes that they had generated. After standards were presented to the group, they were randomized twice; judges were asked about the odor descriptions, and this was discussed and changed if it was needed to fit the consensus of the group about the terms. When the judge was not able to describe the odor correctly, this procedure was repeated. At the end of the session, every judge was able to correctly recognize the odor standard. The judge's performance on the training was monitored by computing standard deviations and variance analysis. Each judge was able to discriminate among the wines and follow the trends of the rest of the panel.

The panel was led through term generation for bell pepper aroma, red fruits, jam, spices, aromatic persistence, and coffee/toasted. These aromas were included on the final scorecard. For each wine aroma standard, two concentrations were prepared as follows: one strong (S) and another with a weak (W) sensation of the odor. Sliced bell pepper in 40 mL of Merlot base wine was used as a standard to train the panel for the bell pepper aroma descriptor (S = 4.0 and W = 2.5 g), while fresh strawberries (S = 4.0 and W = 2.0 g) were used for the red fruits aroma standard, commercial cranberry and blackberry jams (S = 2.5 g of cranberry jam + 2.5 g of blackberry jam and W = 1.0 g of cranberry jam + 1.0 g of blackberry jam) were used for the jam aroma, black pepper (S = 0.08 g and W = 0.03 g) was used for the spices aroma, and a commercial soluble coffee (Nescafé) (S = 0.05 g and W = 0.02 g) was used for generating coffee/toasted aroma descriptors. All of the standards were kept at least overnight at the room temperature before being offered to the panel. It was not necessary to train the panel on the previously selected descriptors by mouth (tannic quality, astringent, alcohol, and equilibrium by mouth). On the training, the panelists were given seven wines per day. Each panelist was asked to individually rate every attribute on the scorecard using a nine-point scale (0 = not present and 9 = extremely intense). On the 2005 vintage sensory testing, the panelists smelled the reference standards to refresh their memories before rating the wines.

GC-MS Analysis. *Reagents and Standards.* Dichloromethane (ultrahigh purity) was purchased from Merck (Darmstadt, Germany); 2-methoxy-3-methyl pyrazine (Aldrich Chemicals Co., Millwaukee, WI) was used as an internal standard. The reagents MIBP (99% pure, Aldrich Chemicals Co.), β-damascenone (77% pure CG, synthesized by Firmenich, Geneva, Switzerland), α-ionone (90% pure), and β-ionone (97% pure) (Aldrich Chemie, Steinheim, Germany) were used as reference standards.

Methodology. The method of Kotseridis et al. (*6*) was used with the following adaptations. Fifty milliliters of wine was added with 50 μL of the internal standard 2-methoxy-3-methylpyrazine at a concentration of 274 μg/L (alcoholic solution, 100% of ethanol) and extracted with dichloromethane. The organic phases were collected, and the stable emulsion was concentrated to 10 times under a nitrogen stream. The analysis was carried out using an Agilent Technologies HP 6890 gas chromatograph Series II fitted with a mass spectrometer detector and equipped with a FFAP capillary column (BP 21, 50 m × 0.32 mm; film thickness, 0.25 μm; SGE, Courtabœuf, France), and an automatic injector (HP 6890 Series Injector) was used. Two microliters of the extract was injected using an automatic sampler in splitless mode. The splitless/split injector was maintained at 250 °C with a flow of 30 mL/min and a split time of 0.5 min. The pressure of the carrier gas (helium 5.6) was 20 psi with a linear velocity of 4.1 mL/min. The temperature of the oven was increased at 4 °C/min from 60 to 220 °C and held at this temperature for an additional 20 min. The detector was maintained at 280 °C. Mass spectra were acquired in the electron impact mode (EI = 70 eV). The mass range was 50−600 m/z, and the electron multiplier was set in the relative mode autotune procedures. The identification of the volatiles compounds in GC-MS was confirmed by comparing both their mass spectra in SCAN mode (NBS75K library) (for β-damascenone) and their retention times in relation to the standards (for α- and β- ionones). The compounds were measured using selected ion monitoring (SIM) mode: ions $m/z = 124$ and 151 for MIBP, $m/z = 124$, 126, and 109 for 2-methoxy-3-methylpyrazine (internal standard), $m/z = 121$ and 192 for α-ionone, $m/z = 177$ for β-ionone, and $m/z = 121$ and 190 for β-damascenone. Ions 124, 121, and 177 were used for quantification, and ions 151, 126, 109, 190, and 192 were used as qualifiers. Calibration graphs, at five or six concentrations levels, were constructed by least-square linear regression using the result for standard solution (12% hydroalcoholic solution). The concentration ranges of the studied compounds were as follows: MIBP, 6.88−68.88 ng/L; α-ionone, 27.60−828.00 ng/L; β-ionone, 24.00−702.00 ng/L; and β-damascenone, 240.00−4800.00 ng/L.

Table 2. Total Acidity (TA), pH, VA, Alcohol (A), and L-Malic Acid (MA) in Cabernet Sauvignon Wines from the Vintages 2004 and 2005

sample wines	pH	TA (tartaric acid g/L)	VA (acetic acid g/L)	MA (g/L)	A (% volume)
		2004 vintage			
774-04	3.80	5.40	0.50	0.48	12.30
960-04	3.80	4.80	0.50	0.08	12.50
1160-04	3.80	5.00	0.50	0.09	13.00
1350-04	3.80	4.80	0.70	0.05	13.50
1415-04	3.70	4.80	0.70	0.11	13.00
		2005 vintage			
774-05	3.80	4.80	0.70	0.06	12.80
960-05	3.70	5.10	0.50	0.14	13.00
1160-05	4.00	4.60	0.50	0.08	13.50
1350-05	3.70	4.90	0.50	0.09	13.60
1415-05	3.70	5.10	0.90	0.04	13.00

Detection and Quantification Limits. A Merlot wine free from MIBP was spiked with 13.78 ng/L of MIBP and submitted to the above-described analysis. For an injection of 2 μL of the wine, the detection limit was the average of three determinations of the lowest measurable peak area plus or minus three and 10 times the standard deviation of this measurement for the detection and quantification limits, respectively.

Repeatability Study. The statistical study was based on six consecutive determinations by six extractions applied to the same wine. The variation coefficients for the analyses ranged from 3 to 12% for individual compounds.

Data Analysis. Analysis of variance (ANOVA), Duncan's Test, PCA, and regression analysis were carried out using Statistica 6 (2001) (StatSotft Inc., Tulsa, OK).

RESULTS AND DISCUSSION

Classical Wine Analyses. The results of pH, total and VA, alcohol, and malic acid concentration of the wines studied are presented in **Table 2**. In general, the wines analyzed had completed malolactic fermentation, with the exception of sample 774-04 (malic acid greater than 0.2 g/L). The VA determination in a wine allows evaluation of the microbiology quality of grapes at the time of collection. The action of *S. cerevisiae* yeast normally permits the formation of 0.2−0.4 g/L of acetic acid in wine. VA is comprised of mainly acetic acid but also lesser quantities of butyric, formic, and propionic acids that were present in every wine, which is almost always bad, having an unpleasant, sharp vinegary aroma. The aroma threshold for acetic acid depends on the wine context and the person's sensitivity of person sniffing, but it is about 0.6−0.9 g/L of wine. The laws in wine-producing countries specify the maximum allowable acetic acid concentration in wine. In Brazil as well as in the European Union and United States, the legal limit of VA in red table wine is 1.2 g/L acetic acid, while in Australia, it is 1.5 g/L. It should be noted that in 1350-04, 1415-04, 1415-05, and 774-05 wines, values above 0.6 g/L were verified. However, these wines did not present an unpleasant smell of acetic acid; overall, for 1415 samples, the main smell sensation was of bell pepper.

Sensory Testing and Descriptive Analysis. Analysis of the data on the intensity scores of each selected sensory descriptor was realized for each wine of the 2004 and 2005 vintages. These were submitted to ANOVA without repeats. When a significant difference was detected, Duncan's test was applied ($p < 0.05$).

Tables 3 and **4** present the mean values of intensity of each selected descriptor for the 2004 and 2005 vintage wines, respectively ($p < 0.05$). The primary difference was the contrast

Table 3. Mean Descriptive Ratings for Sensory Evaluation of the Wines from 2004 Vintage on a Nine-Point Scale, with Corresponding Duncan's Test Values at $p < 0.05$

	mean of sensory ratings					
	774-04	960-04	1160-04	1350-04	1415-04	*p* value[a]
			aroma			
bell pepper	3.9 b	3.1 b	4.1 b	5.7 a	6.3 a	0.000
red fruits	6.6 a	5.0 b	4.7 b	3.7 b	4.4 b	0.007
jam	6.7 a	5.3 a	3.7 b	3.2 b	3.6 b	<0.000
spices	3.4 a	3.8 a	4.2 a	4.8 a	4.9 a	0.111
coffee/toasted	2.4 b	4.5 a	3.2 a,b	3.5 a,b	3.9 a	0.040
			by mouth			
bitter	3.3 c	4.1 b,c	5.4 a	4.7 a,b	5.2 a,b	0.006
astringent	3.2 b	5.0 a	4.4 a	5.1 a	4.8 a	0.009
aromatic persistence	5.2 a,b	5.9 a	4.5 b,c	4.8 b,c	4.0 c	0.004
equilibrium	4.7 a	5.0 a	4.2 a,b	3.4 b,c	3.2 c	0.001

[a] Different letters in the same line are significantly different at the 5% level.

Table 4. Mean Descriptive Ratings for Sensory Evaluation of the Wines from 2005 Vintage on a Nine-Point Scale, with Corresponding Duncan's Test Values at $p < 0.05$

	mean of sensory ratings				
	774-05	1160-05	1350-05	1415-05	*p* value[a]
		aroma			
bell pepper	4.5 a	4.4 a	4.8 a	6.6 b	0.005
red fruits	6.4 a	4.2 b	4.6 b	4.2 b	0.009
jam	1.3 a	2.7 a,b	2.7 b	2.6 a,b	0.014
spices	3.2 a	3.6 a	2.5 a	5.3 b	0.015
coffee/toasted	1.8 a	3.3 a	1.5 a	3.2 a	0.070
		by mouth			
tannic quality	4.6 a	5.5 b	5.6 a,b	5.6 a	0.013
astringent	4.1 b	4.8 a	4.1 a	4.3 a	0.421
aromatic persistence	4.8 a	5.3 b	4.9 a	5.3 b	0.038
equilibrium	4.2 a	5.9 b	4.6 a,b	5.1 a	0.004
Alcohol	4.6 a	5.5 b	4.8 a	5.2 b	0.042

[a] Different letters in the same line are significantly different at the 5% level.

between the vegetal and the berry aromas, and these were the two most highly scored aroma categories in both vintages 2004 and 2005, similar to results for South African and Californian Cabernet Sauvignon wines (*22*, *23*).

In the 2004 vintage, two wines were strongly associated with the bell pepper aroma attribute. These corresponded to wines originating from vineyards cultivated at the highest altitudes of this study (1415-04 = 1415 m and 1350-04 = 1350 m asl). It was noted that wines originating from vines cultivated at lower altitudes (774-04 = 774 m and 960-04 = 960 m asl) were strongly marked by the red fruits aroma and jam aroma attributes. The coffee/toasted aroma distinguished the wines in the low or lower altitude samples. The 774-04 wines had a particularly low astringency in the 2004 vintage, but this attribute was not significant to distinguish the wines in the 2005 vintage. In contrast, the spice aroma was lower for the lower altitude samples. Aromatic persistence was high in 2004 but not in 2005 vintages.

In the 2005 vintage, only wine from the highest altitude (1415-05) was strongly marked by the bell pepper aroma. The contrast between 1415 (bell pepper aroma) and 774 wines (red fruits aroma) was only in the 2004 and 2005 vintages. The jam aroma separated the contradictory mode of 774 wines from the other wine samples, being that more of them were from 2004 than 2005 vintages. A good "equilibrium" was perceived for 774-04 and 774-05 wines.

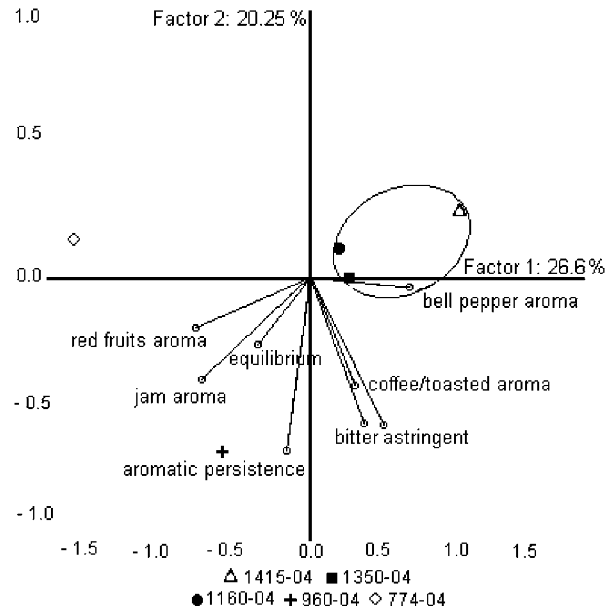

Figure 1. PCA of the wines sensory profile (2004 vintage) using factor 1 × factor 2 (where 1415 = 1415 m, 1350 = 1350 m, 1160 = 1160 m, 960 = 960 m, and 774 = 774 m asl).

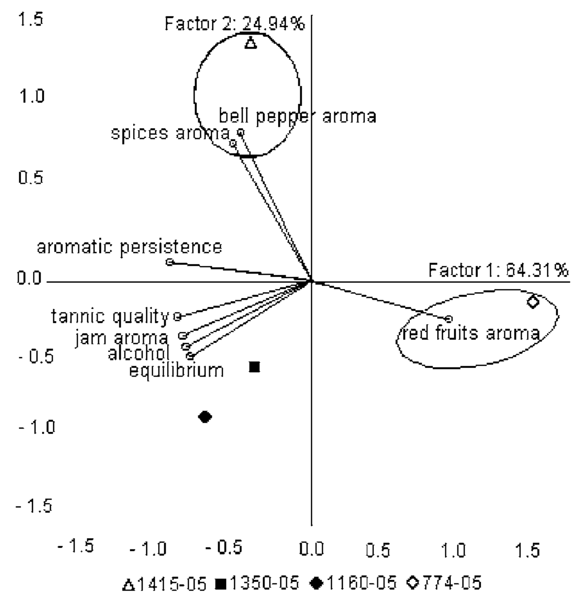

Figure 2. PCA of the wines sensory profile (2005 vintage) using factor 1 × factor 2 (where 1415 = 1415 m, 1350 = 1350 m, 1160 = 1160 m, and 774 = 774 m asl).

PCA was performed on the correlation matrix using the attributes that differed significantly by ANOVA for wines of 2004 and 2005. The judges' scores for the sensory analyses were averaged on the PCA graphs (**Figures 1** and **2**).

Figure 1 presents the axes factor 1 × factor 2 of the PCA regarding the sensory analysis results of the 2004 wines, which explain 46.85% of the total variance among the data. The first axis represents 26.60%, and the second axis represents 20.25% of the total dispersion. The factor 1 axis represents the opposition of red fruits and bell pepper aromas. The factor 2 axis was negatively correlated with bitter, astringent, and aromatic persistence. These data confer with the results obtained in the sensory evaluation of the 2004 vintage, in which wine 1415-04 received the highest scores of the sensory panel in relation to the bell pepper aroma attribute. The wine originating from the vineyard situated at 960 m asl (960-04) was negatively correlated

Table 5. MIBP and Norisoprenoids Levels in Cabernet Sauvignon Wines from Different Altitudes

	concentration (ng/L)[a]									
	2004 vintage					2005 vintage				
compounds	774-04	960-04	1160-04	1350-04	1415-04	774-05	960-05	1160-05	1415-05	cv (%)[b]
MIBP	18.09 b	18.64 b	23.64 b	30.12 a	39.62 a	12.14 c	9.26 b	8.40 b	42.12 a	12
α- ionone	**75.12** a	**162.16** b	**68.20** a	**31.95** c	**66.34** a	**89.11** a	**180.12** c	**13.17** b	**89.36** a	12
β- ionone	195.33 a	**83.22** b	174.42 a	122.05a	144.11 a	142.18 a	98.16 c	114.03 b	139.31 a	6
β-damascenone	16496 a	13331 a	7771 b	7831 b	17196 a	14720 b	14352 b	9483 b	16571 a	3

[a] Means of two repetitions. Different letters in the same line are significantly different at the 5% level (Tukey HSD test). In bold are results below the quantification limits of the method (219 and 96 ng/L for α- and β-ionones, respectively). [b] Method's variation coefficient.

to both of these axes and strongly correlated with the variable aromatic persistence (**Figure 1**). Projection of the cases onto the first two axes shows that the wines originating from higher altitudes (1415-04, 1350-04, and 1160-04) were positively correlated with the bell pepper aroma attribute. Interpretation of these two PCA projections for 2004 vintage Cabernet Sauvignon wines permits the characterization of wines originating from altitudes above 1160 m as vegetative wines, principally characterized by the olfactory attribute bell pepper aroma, and wines originating from the lowest altitude studied (774 m asl) are characterized by the attribute red fruits aroma.

PCA on the sensory scores of the 2005 vintage wines explains the total dispersion of data more satisfactorily than for wines of the 2004 vintage. **Figure 2** presents the PCA factor 1 × factor 2 axes regarding the results of sensory analysis for 2005 vintage wines, which explain 89.25% of the total variance of the data. The first axis represents 64.31%, and the second represents 24.94% of the total dispersion. The factorial coordinates show that the factor 1 axis was strongly positively correlated with the variables red fruits aroma and strongly negatively correlated with the variables jam aroma, spices aroma, tannic quality, aromatic persistence, equilibrium, and alcohol. The factor 2 axis was strongly positively correlated with the variables bell pepper and spices aromas. Projection of the cases onto the variables revealed a strong correlation between wines originating from the highest altitude (1415 m asl) with the factor 2 axis, indicating its direct correlation with the bell pepper aroma attribute, while wine 774 showed a strong correlation to the red fruits aroma attribute. These results are in agreement with the sensory analysis of both vintages.

GC-MS Analyses. *Validation of the Method.* The square of the correlation coefficient of the regression line, obtained from the calibration data for MIBP, α- and β-ionone, and β-damascenone, gave R^2 values varying between 0.9938 (α-ionone) and 0.9981 (MIBP). The repeatability of this method for all of the molecules measured gave presenting variation coefficients of 12, 12, 6, and 3% for MIBP, α- and β-ionone, and β-damascenone, respectively. The detection/quantification limits for all of the compounds measured were as follows: 2.4/7.8, 65.7/219.0, 28.8/95.9, and 160.0/533.3, respectively, for MIBP, α-ionone, β-ionone, and β-damascenone. MIBP quantification was reliable at values as low as 8 ng/L. Because of the sensory detection limit of this molecule in wines (10−16 ng/L) (*16*) and to the rapidity and simplicity of this method and the possibility of MIBP quantification, the use of this method provides advantages for routine analyses, as previously reported by other researchers (*5*, *6*).

Climate Data. The climatic data have been collected by the five meteorological stations for the two vintages (2003/2004 and 2004/2005). Both of the vintages were characterized by an important rainfall index in the vineyards evaluated. The summer of vintage 2003/2004 was strongly marked by a high pluviometric index in the months of December and January for the vineyard at 1415 m asl. In this same vintage, weak precipitations were observed during the maturation period for the vineyard situated at 960 m asl. In general, particularly abundant precipitations were observed between October and January and also in the months of April and May in both vintages. In the 2004/2005 vintages, a strong reduction in the pluviometric index was observed for the vineyard situated at 1415 m asl, especially when compared with the period from October to January of the 2003/2004 vintages. In 2004/2005, the vineyard situated at 774 m asl presented the highest annual rainfall. In relation to temperatures, the months of January and February were particularly hot for the vineyards situated at 774 m, 960 m, and 1160 m asl in both vintages. During the maturation period, minimum temperature values between 10.8 and 16.3 °C were registered.

Chemical, Sensory, and Seasonal Climatic Variation Data and Correlation Analysis. The concentration results of C_{13}-norisoprenoids and MIBP in the wines from the two vintages are presented in **Table 5**. In relation to the norisoprenoid concentrations, the α-ionone concentration found in the wines was lower than the olfactory detection limit of this molecule (400 ng/L) (*17*), and it was always detected below the method quantification limit. Consequently, this compound cannot be considered to be organolepticaly significant in the extracts of Cabernet Sauvignon wines. In contrast, the β-ionone concentration was higher than its olfactory detection limit (90 ng/L) (*11*), and this compound could be a representative aromatic component in the wines odor analyzed, leading them to floral or fruity notes. Regarding the relation between α- and β-ionone, it is interesting to observe that in both vintages, 960 wines samples are the only ones that have higher concentrations of α-ionone than β-ionone. These compound relations can be used for differentiating the Cabernet Sauvignon wines, although more research is still necessary to confirm these data. These compounds can also act in synergy between themselves or with other compounds and present an important role in wine aroma.

β-Damascenone contents found in the Cabernet Sauvignon wines analyzed in our research were higher than the results obtained by Kotseridis et al. (*6*) in Merlot wines (200−1300 ng/L), especially for the wines 774, 960, and 1415; these results were verified in both vintages. Our results were lower than those reported by Simpson and Miller (*9*) and by Guth (*10*). The latter found particularly high β-damascenone concentrations ranging from 66000 to 170000 ng/L in Chardonnay wines (*9*) and 980000 ng/L in Scheurebe wines (*10*). The detection thresholds of β-damascenone vary by a factor from 4000 (*24*) to 50000 ng/L (*9*). It is clear that β-damascenone can have an important impact in the wine aroma.

The warm notes of honey, ripe fruit, and exotic fruits were associated with norisoprenoids in Chardonnay and Sauvignon blanc wines by Tomasi et al. (*14*). In our research, an association between a high concentration of β-damascenone and the highest

Table 6. Climatic Description of Each Season (Winter and Summer) for the Period 2003–2005[a]

	temperature (°C)								rainfall (mm)	
	minimum		maximum		mean		thermal amplitude			
	winter[b]	summer[c]	winter	summer	winter	summer	winter	summer	winter	summer
				to 774 m (774 wines)						
2003/2004	8.8	15.7	22.4	28.7	14.3	20.9	13.6	12.5	514.6	306.4
2004/2005	8.8	16.8	21.2	29.7	13.9	22.3	12.4	12.7	615.6	271.6
				to 960 m (960 wines)						
2003/2004	6.5	13.8	23.5	24.2	12.7	17.9	17.0	10.4	339.4	130.2
2004/2005	10.8	15.8	17.2	24.3	14.5	20.4	6.4	8.5	648.1	466.7
				to 1160 m (1160 wines)						
2003/2004	8.2	12.8	20.2	22.6	11.3	16.7	12.8	9.8	121.9	230.1
2004/2005	8.4	13.7	16.9	25.6	12.7	18.8	8.5	12.0	173.6	368.6
				to 1350 m (1350 wines)						
2003/2004	8.2	14.3	19.7	24.9	13.3	18.6	11.4	10.6	607.8	854.9
2004/2005	7.5	13.1	18.7	23.7	12.5	17.6	11.3	10.6	376.8	1142.8
				to 1415 m (1415 wines)						
2003/2004	7.4	12.1	16.4	21.6	11.0	15.8	9.0	9.3	443.9	278.8
2004/2005	7.1	12.6	15.4	22.9	10.5	16.8	8.3	10.0	475.3	386.2

[a] Values are means for the weather station network near to the vineyards. [b] June, July, August, and September. [c] December, January, February, and March (harvest in March for 774 samples and in April for all of the others samples).

scores of red fruit aroma can be made, except to the wine 1415 wines where this aroma may have been masked by a particularly high concentration of MIBP, responsible for the vegetal aroma (**Tables 3**−**5**).

In the present research, a significant relation between vineyard altitude and α- and β-ionone and β-damascenone concentrations in the wines was not observed ($p > 0.05$), both for the 2004 and for the 2005 vintage (data not shown).

It is known that the pyrazines, chiefly MIBP, strongly contribute to the vegetal bell pepper aroma in Cabernet Sauvignon wines. However, it is important to observe that these pyrazines are not the only compounds responsible for many facets that define a grape or wine as vegetative. Kotseridis and Baumes (*26*) have shown that vegetative notes could also be attributed to aldehydes such as decanal and (*E*,*Z*)-nona-2,6-dienal.

The research has also demonstrated that MIBP concentrations in grapes and wines are influenced by irrigation of the grapevine, variety, season, climate, and sunlight fruit exposure (*1*, *25*). Cabernet Sauvignon wines have been found to have MIBP concentrations in the range of 3.6−56.3 ng/L by Allen et al. (*3*) and approximately 3−36 ng/L by Hashizume and Umeda (*5*).

An important effect of the vintage on the MIBP concentrations in the wines was observed. In the 2004 vintage wines, the MIBP concentrations were greater than the mean olfactory detection limit of this molecule in red wines, 15 ng/L (*16*). For wines 1160-05, 960-05, and 774-05, MIBP concentrations were much lower as compared to the 2004 vintage (**Table 5**). However, for the wine originating from the vineyard situated at the highest altitude, a slight increase in MIBP concentration occurred in the 2005 vintage in relation to that of 2004. It should be highlighted that the 2004 vintage wines presented MIBP concentrations well above the olfactory detection limit and were confirmed as extremely vegetal by the sensory panel, which presented high scores for the bell pepper aroma attribute. In the 2005 vintage, MIBP concentrations were above the olfactory detection limit in only one of the four wine samples analyzed (1415-05 wines). MIBP contents in wines have always shown a consistent relationship between winegrape variety and grape-growing conditions. Its presence has been associated with a lack of ripeness of grapes (*16*). However, according to the maturity analyses carried out in the grapes at the moment of harvest (data not shown), commercial maturation of Cabernet Sauvignon has been well-reached, with grapes presenting high total solid soluble (TSS) values (range between 20.3 and 23.5 °Brix), low titratable acidity, and maturation indexes (TSS/acidity ratio between 25 and 38). Consequently, all of the wines have presented a high alcohol content (**Table 2**). Thus, we can suggest that the presence of MIBP in these wines was not related to with a lack of grape ripeness. MIBP is stable after vinification; it persists in the wine during its aging. However, the actual methoxy pyrazine precursors have not been definitively determined. It is found in the prevéraison berry, but it is not known whether these pyrazines are formed in the berry or whether they are transported to the berry from the vine vegetative structures (*16*). Research remains to be done on a complete understanding of the presence of pyrazines in grapes.

Climatic data from the summer and winter seasons of the 2003/2004 and 2004/2005 vintages were associated to the sensory analysis results of both vintages. The mean data of minimum, maximum, and mean temperatures, thermal amplitude, and rain incidence (mm) are presented in **Table 6**.

Analyzing the climatic data revealed that in the 2004, the highest temperatures occurred in the summer in the vineyards situated at 774 and 960 m asl; samples originating from these vineyards were more readily associated with red fruits and jam aromas (**Table 3**). In 2005, despite the considerable increase in rain volume in winter and a slight decrease in thermal amplitude in relation to the previous year, observation revealed that similar climatic conditions were recorded for the vineyard situated at 774 m asl, accompanied by a strong association of wine from this region with the red fruits aroma, but not with jam aroma attribute for this vintage (**Table 4**).

The wines originating from the vineyard situated at 1415 m asl in both the 2004 and the 2005 vintages were strongly associated with the bell pepper aroma attribute. Observation showed wines that received the highest scores for this attribute also received higher scores for spices aroma (**Tables 3** and **4**). The results of MIBP concentration analysis point to the fact that in the greatest altitude (1415 m asl), the temperature was lower in relation to the remaining regions studied, both in winter

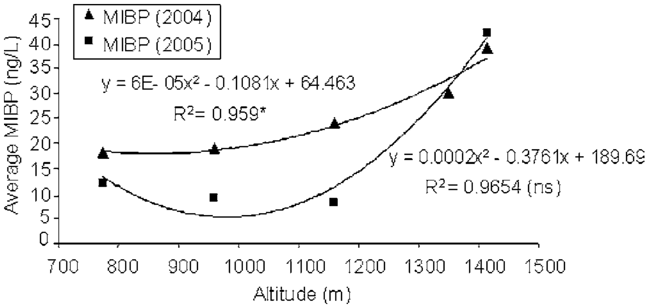

Figure 3. Concentration of MIBP, from 2004 and 2005 vintages, in response to vineyard altitude. *Indicates significance at $p < 0.05$.

and in summer, and the MIBP concentration was clearly greater in relation to wines originating from the other regions. These results agree with studies realized on Cabernet Sauvignon and Sauvignon blanc varieties, where the concentration of methoxypyrazines was greater in grapes cultivated in cooler climatic conditions (*1*, *7*). Prior research indicates that lower temperatures related to the period that antecedes véraison more significantly influence the MIBP concentration in grapes than after their maturation (*7*).

For the 2004 vintage, with the exception of the wines originating from two vineyards situated at the highest altitudes (1415-04 and 1350-04 wines), the remaining wines were not strongly characterized by the sensory bell pepper attribute. However, the results of the quantitative analysis of MIBP showed values above the olfactory detection limit of this compound in all of the red wines analyzed in this vintage (>15 ng/L), thus indicating that the sensory panel only identified as bell pepper aroma, wines containing greater MIBP concentrations, and were comparatively unable to identify lower concentrations (**Tables 3−5**). Allen et al. (*2*), while evaluating Australian Sauvignon blanc wines with increasing concentrations of added MIBP, observed similar results. The authors verified that the sensory panel, composed of judges experienced in the evaluation of these wines, was capable of distinguishing the aroma of bell pepper only in wines with a relatively high quantity of MIBP (26.9−33.9 ng/L) but was unable to distinguish the same in wines with lower quantities (4.7−12.0 ng/L).

In both vintages, the wines originating from vineyards situated at 1160 and 1350 m asl were characterized by the attributes aromatic persistence and equilibrium. The two tasting descriptors tannic quality and alcohol, previously added to the sensory descriptors of the 2005 vintage wines, also presented greater sensory scores for these wines.

With the aim of correlating the chemical analyses results of these wines with vineyard altitude, regression analyses were realized. Because the MIBP concentration in the grape is intimately related with viticulture conditions, such as growing temperature (*3*, *7*), research aimed at relating vineyard altitude with MIBP concentrations is required. Second-degree polynomial models were applied to the results of MIBP concentrations (ng/L) in the wines, as a function of vineyard altitude for the 2004 and 2005 vintages. A high determination coefficient was verified in both vintages, although only significant for the 2004 vintages ($p < 0.05$) (**Figure 3**). The 1350 wine sample of 2005 vintage (1350-05) was lost during the samples transport from Santa Catarina to Bordeaux. Probably the lack of 1350-05 samples made it difficult to determination the coefficient result significance for the relationship between MIBP and altitude of 2005 vintage.

Table 7. Correlation Analysis among Temperature Data Set, Vineyard Altitude, and MIBP Content in the Wines

MIBP content	MIBP content	vineyard altitude	minimum temp	maximum temp	average temp	thermal amplitude
vineyard altitude	0.775					
minimum temp	−0.850	−0.956				
maximum temp	−0.863	−0.979	0.976			
average temp	−0.827	−0.681	0.735	0.772		
thermal amplitude	−0.864	−0.978	0.986	0.998	0.775	

Correlation analyses with data of the two vintages confirm that the MIBP content in the wines was positively correlated with altitude vineyard and strongly negatively correlated with seasonal temperature data (**Table 7**). A strong negative correlation was also observed between the vineyard altitudes with seasonal temperature values. This observation is important because it shows that in the highest vineyards both winter and summer seasons are cooler.

In conclusion, considering the results of the descriptive and sensory analyses, the wines originating from vineyards situated at the highest altitude studied (1415 m asl) were strictly associated with the sensory attribute bell pepper aroma, while wines produced at the lowest altitude (774 m asl) were considered more closely associated with the attribute red fruits aroma. An insignificant relation was observed between α- and β-ionone and β-damascenone concentrations in the wines and the vineyard altitudes studied. It is, however, clear from these results that seasonal climate is not solely responsible for the wine aroma characteristics, and the influence of other factors in these sites, such as soil attributes and sunlight, should be investigated in future researches. It is worth emphasizing the importance of characterizing the terroir as a whole to more clearly understand the characteristics of the Cabernet Sauvignon wine recently produced in this region, which presents distinct climatic and orographic conditions from the remainder of Brazil.

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