



Red wine produced from the Isabella and Ives cultivar (*Vitis Labrusca*): profile of volatiles and aroma descriptors

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Abstract

Considering the potential consumption and economic the importance that Isabella and Ives wines represent in the Brazilian consumer market as well as the scarcity of scientific data examining their quality, the objective of this study was to investigate the sensory quality and the volatiles profile of these wines. The volatile compounds were extracted by headspace solid-phase microextraction (HS-SPME) and a total of 54 compounds were detected in red wine samples including esters (23), terpenes (12), alcohols (10), aldehydes and ketones (5) and amines (1) as well as 3 compounds belonging to other classes. Isabella and Ives red wines were sensorially characterized by 14 descriptors, through quantitative descriptive analysis (QDA). The PCAs fruity descriptors were the primary contributors to the aroma profile of the analyzed wines due to the presence of ethyl acetate and esters, especially in the wine coded as QM, which exhibited the highest variety of compounds. The differences observed in the principal components analysis, might have been influenced by the grape composition of each wine. Although the wines were from the same region, each came from a different winery and was subject to unique production processes.

Keywords: aroma enology; extraction; fermented drink; mass spectrometry.

Practical Application: Data of volatile compounds profile and aroma descriptors of red wines are of great importance in understanding the positive and negative aromatic quality of wines that direct affects the acceptance and/or rejection of wines by the consumers. Futhermore, it is important to emphasize that these results contribute to enhancing scientific knowledge on aromatic quality of red wines produced with Brazilian Isabella and Ives cultivars.

1 Introduction

Aroma is the most important attribute influencing wine quality. Aroma can induce flavor sensations and can be used to identify compounds that might aid in the development of higher quality wines (Coetzee et al., 2015; Welke et al., 2012).

For consumers, the ability to recognize aroma in wine can be influenced by several factors, the most important of which are emotional state, training and physiology (Castilhos et al., 2012). In a competitive consumer market, the concept of quality is entirely based on satisfying consumer expectations, and opposing expectations compromise product success in the consumer market (Castilhos et al., 2012).

The production and consumption of wine made from American cultivars, especially *Vitis labrusca* and/or hybrid cultivars, known as “table wines” or “common wines,” is higher than those of wine made with *Vitis vinifera*. For example, in 2016, the Brazilian production of table wine was 247,457,542.0 million liters, 85.0% of which were table wines made from American cultivars (Biasoto et al., 2014; Castilhos et al., 2012; Mello, 2015). These wines are produced in high quantities because in several regions of Brazil, the weather conditions are unfavorable for *V. vinifera* grape cultivation, whereas American or hybrid

cultivars are highly adaptable to adverse weather conditions and show greater resistance to diseases (Rombaldi et al., 2004; Brasil, 2014).

The wine produced with American cultivars, especially using the Isabella and Ives are identified as having a grape aroma and a raspberry flavor (Rizzon et al., 2000; Rombaldi et al., 2004). Despite its aromatic characteristics, Isabella and Ives wines are one of the most popular in the country because of the habits of consumers and the health benefits of tannins and pigments (Rizzon et al., 2000; Rombaldi et al., 2004).

A review in the scientific literature found a few studies that investigated the profile of volatile and sensory compounds in red wine produced with Isabella and Ives cultivars. Biasoto et al. (2010, 2014) explored aspects related to the sensory quality and consumer preference of wine made from *V. labrusca* and its hybrids, including the Isabella and Ives cultivar; however, data on sensory quality and its relation to volatiles are lacking. Our group has carried out studies on the physicochemical composition (Arcanjo et al., 2017) and the extraction process of the volatile compounds by HS-SPME-GC/MS technique (Arcanjo et al., 2015) of red wine produced with *V. labrusca*

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cultivars. Thus, considering the potential consumption and economic importance that Isabella and Ives wines represent in the Brazilian consumer market as well as the scarcity of scientific data examining its quality, the objective of this study was to investigate the sensory quality and the volatiles profile of wine produced from Isabella and Ives cultivars, the most prolific *V. labrusca* cultivar in traditional wineries from the South Region of Brazil.

2 Materials and methods

2.1 Wine samples

The aromatic quality of three dry red Isabella and Ives cultivars wines produced in traditional wineries in Southern Brazil was evaluated (Table 1). The data were obtained from five different bottles of wines from the same lot and the experiments were performed in triplicate. The wines were stored at 10 °C prior to analysis.

2.2 Aroma descriptors analysis of Isabella and Ives red wine

The aroma profiles were generated by a trained panel of 8 judges, aged between 20 and 40 years who were undergraduate, graduate students and employees of the Federal University of Paraíba (Universidade Federal da Paraíba), Brazil, and had experience in wine consumption using quantitative descriptive analysis (Meilgaard et al., 1999; Stone et al., 1974; Stone and Sidel, 2004). The judges were initially tested based on: (i) the ability to express proportionality using the scales test, (ii) the sensitivity to recognize and perceive differences in aroma between the samples, and (iii) the ability to identify 32 odors present in the Wine Aroma Wheel (Noble et al., 1987).

Descriptive terminology development and team training

The judges determined the descriptive terminology for the red wines using the Repertory Grid Keily's Method described by Moskowitz (1983). Red wine samples were grouped into pairs, and the judges were asked to evaluate the similarities and differences regarding the aroma attributes. The analysis was performed individually first, then with the help of a leader, and the terms were discussed using descriptive synonyms and antonyms. Subsequently, the terms were grouped and/or eliminated by consensus, and those that best described the similarities and differences between the wines were selected. This process generated 14 aroma descriptors. The final descriptive team was selected as the flavors who exhibited good discriminative power ($pF_{\text{sample}} \leq 0.05$), good reproducibility in judgment ($pF_{\text{replicates}} \geq 0.05$)

and consensus with the team for at least 80% of the descriptors on the sheet generated by team consensus (Damásio, 1991).

Final evaluation of Isabella and Ives red wine

The dry red wines (30 mL) were presented in tulip glasses covered with a watch glass and assigned random three-digit numbers. The wines were kept at 20 ± 2 °C and were evaluated in individual cabins with white light. Data were collected using the descriptive sheet generated by panel consensus, which contained 9-cm non-structured scales with terms expressing intensity, from "none" at one end of the scale to "intense" at the other end. Each judge evaluated each wine in two sessions, with three triplicates per session, totaling six replicates.

2.3 Extraction and chromatographic analyses to identify volatile compounds

The volatile compounds were extracted by headspace solid-phase microextraction (HS-SPME) (Arcanjo et al., 2015). The fiber used was polydimethylsiloxane-carboxen-divinylbenzene (PDMS/CAR/DVB) (Supelco, Bellefonte, PA, USA). For this purpose, 30 mL of red wine were added to a 100-mL screw cap glass flask with a septum. The volatile compounds were extracted by placing the flask in a 30 °C water bath with internal magnetic stirring. The sample reached equilibrium in 15 minutes and was then exposed to the fiber for 35 minutes.

Volatile compounds of red wine were separated, a Varian Saturn 2000R 3800 gas chromatograph (GC) coupled to a Varian Saturn 2000R 2000 mass detector and a VF-5MS column (60 m × 0.25 mm × 0.25 μm) was used. The carrier gas was helium at a 1.0 mL minute⁻¹ flow rate. The samples were injected by placing the SPME fiber at the entrance of the GC at 250 °C and the injection mode were split (1:10) with a desorption time of 10 minutes. The initial oven temperature was 35 °C, which was maintained for 5 minutes, increased to 240 °C at 3 °C per minute, and then maintained at 240 °C for 5 minutes. The mass spectrometer was operated in the electron impact mode with a source temperature of 200 °C, an ionization energy of 70 V and scan variation of m/z 29 to m/z 400 at 3.33 scans/s.

The mass spectra of unknown volatile compounds were also compared with standard compounds using the GC/MS library spectra bank, the NIST/EPA/NIH Mass Spectral Database (Version 1.7) and existing databases in the literature. The identification was aided by comparing the linear retention indices (LRI) obtained with a standard homologous n-alkane solution of the 0,04 mg.mL⁻¹ (C7-C30) injected to obtain the linear retention times of each compound.

Table 1. Characterization of the dry wine samples produced in 2012, the origin of production and the price per bottle.

Region	Code ¹	Varietals ²	Origin	Price ³
South	QM	Isabella, Ives	Flores da Cunha-RS	8.65 (2.54)
	SB	Isabella, Ives, Seibel and Concord	Bento Gonçalves-RS	7.79 (2.28)
	MR	Isabella, Ives	Flores da Cunha-RS	8.45 (2.48)

¹ Codes for the analyzed wines; ² Varietals each analyzed wine contains; ³ Sale price in R\$ (US\$) (dollar exchange rate R\$3.41 on 15/05/2017) per 750 mL.

2.4 Quantification of higher alcohols, acetaldehyde and ethyl acetate by GC

The volatile compounds acetaldehyde, ethyl acetate, methanol, 1-propanol, 2-methyl-1-propanol, 2-methyl-1-butanol and 3-methyl-1-butanol (Sigma-Aldrich, Brazil, 2014) were the secondary compounds of alcoholic fermentation and quantified by gas chromatography according to methodology proposed by the Brazilian Ministry of Agriculture, Livestock and Food Supply (Ministério da Agricultura, Pecuária e Abastecimento – MAPA) (Brasil, 2005; Rizzon, 2010). A gas chromatograph (VARIAN 430-GC, California, USA) coupled to a flame ionization detector (FID) with a fused silica capillary column (CP WAX 52 CB, VARIAN, California, USA) measuring 60 m x 0.25 mm x 0.25 µm was used. Helium was used as the carrier gas (flow rate of 1 mL minute⁻¹). The initial oven temperature was 50 °C for 5 minutes and was programmed to reach 170 °C by increasing 6 °C/min, then increased to 240 °C at a rate of 30 °C/min and remained at this temperature for 2 minutes. The samples (1 µL) were injected after adding 10% by volume of an internal standard solution (4-methyl-2-pentanol at 1 g L⁻¹) at a split of 1:100. The chromatograms were recorded in the Galaxie Chromatography Data System software.

The concentration of each substance in the wines was obtained using Equation 1:

$$C = c * (h * I) / (H * i) \quad (1)$$

in which C is the concentration of the substance in the wine in mg L⁻¹, c is the concentration of the substance in the standard solution, h is the peak height of the substance in the wine, H is the peak height of the substance in the standard solution, I is the peak height of the internal standard in the standard solution and i is the peak height of the internal standard of the substance in the wine.

2.5 Statistical Analysis

The results were analyzed using analysis of variance (ANOVA) and Tukey's mean test at 5% significance using Statistica 5.0 (StatSoft Inc., 2001). Data from the sensory analysis and volatiles profile were also evaluated by principal component analysis (PCA) using XLSTAT version 5.03 (Addinsoft, ESPAÑA, 2014), and a Pearson's linear correlation analysis was performed with data from the sensory analysis using SPSS (IBM SPSS Statistics, version 24.0)

3 Results and discussion

3.1 Sensory analysis

The wines produced with Isabella and Ives cultivars were characterized and described according to fourteen sensory attributes (Table 2): floral, tropical fruits, ripe fruits, hot, red fruits, ripe grape, woody, licorice, honey, spices, molasses are considered positive attributes and pungent, volatile acidity and oxidized are negatives attributes.

The wines produced with Isabella and Ives cultivars had a very similar aroma sensory profile as perceived by the judging team. Significant differences between the wines (p<0.05) were detected only in four aroma descriptors: pungent, tropical fruits, ripe grape

and licorice. According Jofré et al. (2006), Verzera et al. (2008) and Baiano et al. (2017) the wine aroma is influenced by many factors: the vineyard ecosystem (soil, microclimate and macroclimate, canopy) and its management are important in definition of grapes and, consequently, of wines characteristics. Thus, even without knowing the production process of the wines analyzed, these differences were perceived between the SB, QM and MR wines, which might be a result of each wine's varietal composition and other factors associated with the wine production process.

The fruity aromas received higher intensity scores in the sensory evaluation of Isabella and Ives red wines, which was also observed by Biasoto et al. (2014), in which all of the analyzed wines containing the *V. labrusca* grape and its hybrids, especially wines containing the Ives and Isabella and Ives cultivars, exhibited greater (p≤0.05) scores for descriptors such as "grape juice," "grape" and "sweet." Another study has also reported that *V. labrusca* varietals such as Ives, Concord, Isabella and Niagara, among others, as containing methyl anthranilate, an ester of phenol derivatives that confers a fruity and/or artificial grape aroma to wine (Reynolds et al., 2005).

3.2 Determination of volatile compounds

Determination of volatile compounds using the HS-SPME-GC/MS technique

The volatile compounds in Isabella and Ives cultivars wines identified by SPME-CG-MS are shown in Table 3. A total of 54 volatile compounds were identified, including esters (23), which were the most numerous, followed by terpenes, (12), alcohols (10), aldehydes and ketones (5), amines (1), and three (3) volatile compounds that were identified as belonging to other classes. Some volatiles that were detected with larger peak values in red Isabella and Ives cultivars wines include 1-propanol, phenylethyl alcohol, butyric acid, 2-methylbutyl ester, ethyl hexanoate, diethyl succinate, ethyl decanoate, α-terpineol and ionone.

Table 2. Mean scores provided by the sensory team for the aroma attributes characterizing Isabella and Ives grape wines.

ATTRIBUTE	WINE		
	QM	SB	MR
Floral	3.3 ± 13.3 ^a	3.4 ± 22.8 ^a	4.0 ± 16.1 ^a
Pungent	2.2 ± 33.7 ^a	2.4 ± 38.4 ^a	2.3 ± 36.2 ^b
Tropical Fruits	0.3 ± 82.8 ^b	1.3 ± 75.4 ^a	0.4 ± 87.1 ^b
Ripe Fruits	2.6 ± 37.8 ^a	2.8 ± 29.3 ^a	3.3 ± 45.6 ^a
Hot	2.2 ± 53.6 ^a	1.4 ± 68.7 ^a	1.3 ± 42.0 ^a
Red Fruits	3.4 ± 32.0 ^a	3.2 ± 33.7 ^a	3.2 ± 27.7 ^a
Ripe Grape	3.8 ± 23.1 ^{a,b}	2.8 ± 22.1 ^b	4.0 ± 27.2 ^a
Woody	2.8 ± 48.9 ^a	2.3 ± 59.0 ^a	2.3 ± 51.8 ^a
Licorice	5.1 ± 14.1 ^a	3.8 ± 38.2 ^b	4.8 ± 31.0 ^{a,b}
Molasses	3.1 ± 56.5 ^a	1.4 ± 62.7 ^a	2.1 ± 46.1 ^a
Honey	0.5 ± 39.1 ^a	1.0 ± 25.9 ^a	0.8 ± 35.5 ^a
Spices	0.4 ± 60.5 ^a	0.4 ± 57.4 ^a	0.3 ± 80.1 ^a
Volatile Acidity	0.6 ± 1.5 ^a	0.4 ± 1.0 ^a	0.4 ± 0.9 ^a
Oxidized	0.1 ± 1.0 ^a	0.2 ± 1.0 ^a	0.1 ± 1.0 ^a

QM (Isabella and Ives wine), SB (Isabella, Ives, Seibel and Concord wine), MR (Isabella and Ives wine). ^{a,b} Different letters in the column denote statistically difference between means from attributes (p < 0.05).

Table 3. Volatile compounds identified by GC/MS and secondary volatile compounds quantified (mg L⁻¹) in red wine produced from the Isabella and Ives cultivar.

Volatile Compound (SPME-GC/MS)	Description ¹	Peak Area Count ^{2,3} ± SD				LRI (lit) ³	Identification method ⁴
		QM	SB	MR	LRI (cal)		
Alcohol							
1-Propanol	alcoholic, pungent	8.89. 10 ⁶ ± 0.6. 10 ^{6a}	2.47. 10 ⁶ ± 0.6. 10 ^{6b}	3.94. 10 ⁶ ± 0.6. 10 ^{6b}	<600	548	MS
7-Octen-2-ol, 2,6-dimethyl-		6.69. 10 ⁶ ± 0.4. 10 ⁶	nd	nd	<600	-	MS
1-Butanol, 2-methyl		3.60. 10 ⁶ ± 0.6. 10 ⁶	nd	nd	751	728	MS+LRI
1-Butanol (Butyl alcohol)	alcoholic	4.96. 10 ⁵ ± 0.5. 10 ⁵	nd	nd	778	665	MS+LRI
2,3-Butanediol (Dimethylethylene glycol)	chemical	7.03. 10 ⁴ ± 0.4. 10 ⁴	nd	nd	817	743	MS+LRI
1-hexanol	fragrant, floral	nd	4.49. 10 ⁴ ± 0.4. 10 ⁴	nd	881	861	MS
3-Nonen-2-ol		6.18. 10 ⁴ ± 0.4. 10 ⁴	nd	nd	929	-	MS
1,6-Octadien-3-ol, 3,7-dimethyl- (Linalool)	citric, floral, floral citrus fragrant	1.39. 10 ⁵ ± 0.5. 10 ^{5b}	nd	8.67. 10 ⁵ ± 0.5. 10 ^{5a}	1110	1091	MS+LRI
Phenylethyl Alcohol	woody (woody-rose-honey), floral	3.63. 10 ⁶ ± 0.6. 10 ^{6a}	2.31. 10 ⁶ ± 0.6. 10 ^{5a}	4.22. 10 ⁶ ± 0.6. 10 ^{5a}	1137	1098	MS+LRI
1-Nonanol	citronella oil, citrus	7.73. 10 ⁵ ± 0.5. 10 ^{5a}	nd	5.31. 10 ⁵ ± 0.5. 10 ^{4a}	1186	1163	MS+LRI
Esters							
Propanoic acid, ethyl ester (Ethyl propanoate)	sweet, fruity	2.84. 10 ³ ± 0.4. 10 ^{3b}	3.59. 10 ⁴ ± 0.4. 10 ^{3b}	9.65. 10 ⁵ ± 0.5. 10 ^{5a}	718	688	MR+LRI
Butanoic acid, 2-methylbutyl ester	fruity	7.81. 10 ⁶ ± 0.6. 10 ^{6a}	nd	nd	749	1044	MS
Propanoic acid, 2-methyl-, ethyl ester (Isobutyric acid, ethyl ester)	fruity, apple, banana, pineapple	3.89. 10 ⁵ ± 0.5. 10 ^{5a}	1.78. 10 ⁵ ± 0.5. 10 ^{3a}	1.81. 10 ⁵ ± 0.5. 10 ^{4a}	764	745	MS+LRI
1-Pentanol, 2-methyl-, acetate		nd	4.34. 10 ⁵ ± 8.50	nd	779	984	MS
Butanoic acid, ethyl ester (Ethyl butyrate)	banana, pineapple	3.06. 10 ⁵ ± 0.5. 10 ^{5a}	1.04. 10 ⁵ ± 0.5. 10 ^{4a}	2.16. 10 ⁵ ± 0.5. 10 ^{4a}	801	787	MS+LRI
2-Butenoic acid, ethyl ester;		6.22. 10 ⁴ ± 0.4. 10 ^{4b}	3.88. 10 ⁴ ± 0.4. 10 ^{4b}	1.89. 10 ⁵ ± 0.5. 10 ^{4a}	854	827	MS+LRI
Butanoic acid, 2-methyl-, ethyl ester	fruity, sweet, apple, banana, grape	2.68. 10 ⁴ ± 0.4. 10 ^{4b}	7.65. 10 ⁴ ± 0.4. 10 ^{3a}	6.11. 10 ⁴ ± 0.4. 10 ^{3ab}	855	839	MS+LRI
Butanoic acid, 3-methyl-, ethyl ester (Ethyl isovalerate)	herbaceous, pineapple, grape, fruity, sweet	5.39. 10 ⁵ ± 0.5. 10 ^{5a}	1.71. 10 ⁵ ± 0.5. 10 ^{4b}	1.08. 10 ⁵ ± 0.5. 10 ^{4b}	859	840	MS+LRI
1-Butanol, 3-methyl-, acetate (Isoamyl acetate)	banana	6.57. 10 ⁵ ± 0.5. 10 ^{5a}	3.54. 10 ⁵ ± 0.5. 10 ^{4b}	2.60. 10 ⁵ ± 0.5. 10 ^{4b}	883	861	MS+LRI
1-Butanol, 2-methyl-, acetate		1.34. 10 ⁵ ± 0.5. 10 ^{4a}	7.14. 10 ⁴ ± 0.4. 10 ^{2ab}	nd	885	868	MS+LRI
Hexanoic acid, ethyl ester (Ethyl hexanoate)	fruity	4.81. 10 ⁶ ± 0.6. 10 ^{6a}	2.35. 10 ⁶ ± 0.6. 10 ^{5a}	2.52. 10 ⁶ ± 0.6. 10 ^{6a}	1005	985	MS+LRI
Hex-4-enoic acid, ethyl ester		3.34. 10 ⁵ ± 0.5. 10 ⁵	nd	nd	1008	-	MS
2-Hexenoic acid, ethyl ester	herbaceous, floral, sweet anise	1.46. 10 ⁵ ± 0.5. 10 ^{4a}	7.47. 10 ⁴ ± 0.4. 10 ^{3b}	nd	1055	1040	MS
Geranylvinyl ether		nd	8.41. 10 ⁴ ± 0.4. 10 ³	nd	1070	1082	MS+LRI
Propanoic acid, 2-hydroxy-, 3-methylbutyl ester (Isoamyl lactate)	lactic, raspberry	nd	6.54. 10 ⁴ ± 0.4. 10 ⁴	nd	1082	1084	MS+LRI
Heptanoic acid, ethyl ester	grape, fruity	3.39. 10 ⁵ ± 0.5. 10 ^{5a}	6.66. 10 ⁴ ± 0.4. 10 ^{4a}	1.24. 10 ⁵ ± 0.5. 10 ^{4a}	1105	1111	MS+LRI
Butanedioic acid, diethyl ester (Succinic acid, diethyl ester)	fruity, apple, strawberry	4.37. 10 ⁶ ± 0.6. 10 ^{6a}	1.54. 10 ⁶ ± 0.6. 10 ^{5a}	3.56. 10 ⁶ ± 0.6. 10 ^{5a}	1170	1149	MS+LRI
Octanoic acid, ethyl ester (Ethyl caprylate)	apple, vinous	1.38. 10 ⁵ ± 0.8. 10 ^{4a}	1.44. 10 ⁵ ± 0.5. 10 ^{4a}	nd	1180	1224	MS+LRI
Benzenoacetic acid, ethyl ester		nd	6.60. 10 ⁴ ± 0.4. 10 ⁴	nd	1270	-	MS
Decanoic acid, methyl ester		1.45. 10 ⁵ ± 0.5. 10 ⁴	nd	nd	1333	1382	MS+LRI
Decanoic acid, ethyl ester (Ethyl decanoate)	fruit, oily, floral	7.46. 10 ⁶ ± 0.6. 10 ^{6a}	1.69. 10 ⁶ ± 0.6. 10 ^{6b}	3.09. 10 ⁶ ± 0.6. 10 ^{6b}	1398	1583	MS+LRI
Dodecanoic acid, ethyl ester (Ethyl dodecanoate)	fruit, oily, floral	1.36. 10 ⁵ ± 0.5. 10 ^{4a}	nd	1.40. 10 ⁵ ± 0.5. 10 ^{4a}	1590	1591	MS
Hexadecanoic acid, ethyl ester (Ethyl hexadecanoate)		nd	nd	5.77. 10 ⁴ ± 0.4. 10 ^{4b}	1861	1990	MS

¹ Aroma description obtained from an online database available at The LRI & Odour Database (2017), Jewison et al. (2012) and Jiang et al. (2013); ² Peak area count values, Mean ± Standard Deviation of the triplicate injection of samples; ³ LRI (literature) from Jiang et al. (2013) and Waldegeris et al. (2011); ⁴ MS ± LRI, mass spectrum and LRI according to a compound run in the VF-5 MS column, and the MS mass spectrum according to the reference spectrum in the NIST/EPA/NIH database and LRI that agreed with the literature. nd: Compound not identified; ⁵ Mean ± Standard Deviation of secondary volatile compounds quantified using the straight-line equation obtained from the injection of a standard curve of compounds into a gas chromatographer with flame ionization detector. ^{a-b} Different letters in the column denote statistically difference between means from attributes (p < 0.05). QM (Isabella and Ives), SB (Isabella, Ives, Seibel and Concord wine), MR (Isabella and Ives wine).

Table 3. Continued...

Volatile Compound (SPME-GC/MS)	Description ¹	Peak Area Count ^{2,3} ± SD				LRI (lit) ³	Identification method ⁴
		QM	SB	MR	LRI (cal)		
Terpenes							
Styrene (Cinnamol)	sweet, cinnamon	2.62. 10 ⁵ ± 0.5. 10 ^{4a}	3.89. 10 ⁴ ± 0.4. 10 ^{3b}	nd	903	890	MS
1-Methyl-4-(1-methylethylidene)-1-cyclohexene (Terpinolene)	anise (fennel) oil, mint	3.65. 10 ⁵ ± 0.5. 10 ^{5a}	9.39. 10 ⁴ ± 0.4. 10 ^{4a}	2.52. 10 ⁵ ± 0.5. 10 ^{5a}	1028	1089	MS+LRI
o-Cymene	thyme essential oil	1.12. 10 ⁵ ± 0.5. 10 ^{5a}	1.44. 10 ⁵ ± 0.5. 10 ^{4a}	nd	1037	1022	MS+LRI
Cyclohexene, 1-methyl-4-(1-methylethyl), (Limonene)	citric, floral, green	2.63. 10 ⁵ ± 0.5. 10 ⁵	nd	nd	1041	1028	MS+LRI
2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene (α-Pinene)	nd	5.52. 10 ⁴ ± 0.4. 10 ⁴	nd	nd	1042	980	MS
Eucalyptol	eucalyptus oil	8.99. 10 ⁴ ± 0.65. 10 ^{4a}	5.83. 10 ⁴ E ± 0.4. 10 ^{3a}	9.04. 10 ⁴ ± 0.9. 10 ^{3a}	1047	1030	MS+LRI
4-Terpinenyl acetate	nd	5.85. 10 ⁴ ± 0.4. 10 ^{4a}	3.79. 10 ⁴ ± 0.4. 10 ^{3a}	nd	1048	1270	MS+LRI
α-Terpinene	citrus, lemon, lime	3.27. 10 ⁵ ± 0.5. 10 ⁵	nd	2.66. 10 ⁶ ± 0.6. 10 ⁵	1098	1015	MS
3-Cyclohexene-1-methanol, α,α,4-trimethyl-, propanoate (α-Terpinyl propionate)	nd	nd	nd	nd	1214	-	MS
α-Terpineol	lilac lily of the valley (floral)	3.46. 10 ⁵ ± 0.5. 10 ^{3a}	9.58. 10 ⁴ ± 0.4. 10 ^{4a}	3.58. 10 ⁴ ± 0.5. 10 ^{3a}	1224	1285	MS+LRI
3-Cyclohexene-1-methanol, α,α,4-trimethyl-, acetate (α-terpineol, acetate)	herbal, spicy, woody, floral,	5.04. 10 ⁵ ± 0.5. 10 ^{5a}	1.03. 10 ⁵ ± 0.5. 10 ^{4b}	9.56. 10 ⁴ ± 0.4. 10 ^{3b}	1264	-	MS
Naphthalene, 1,2-dihydro-1,1,6-trimethyl-	yeast	1.05. 10 ⁵ ± 0.5. 10 ^{4b}	6.12. 10 ⁵ ± 0.5. 10 ^{5a}	nd	1392	1336	MS+LRI
Aldehydes and Ketones							
Methylglyoxal (Pyruvaldehyde)	acid-sweet	4.50. 10 ⁴ ± 0.4. 10 ⁴	nd	nd	<600	-	MS
Butanal, 3-hydroxy (Acetaldo)	nd	nd	3.06. 10 ⁵ ± 0.5. 10 ⁵	nd	<600	-	MS
2-Propanone, 1,1-dithoxy-, (Pyruvaldehyde, 1-(diethylacetal))	nd	nd	7.52. 10 ⁴ ± 0.4. 10 ⁴	nd	731	644	MS+LRI
3-Hepten-2-one	fruity, pineapple, passion fruit	6.87. 10 ⁴ ± 0.4. 10 ³	nd	nd	794	-	MS
Ionone	rose oil	3.48. 10 ⁶ ± 0.6. 10 ^{5a}	1.53E. 10 ⁶ ± 0.6. 10 ^{5b}	2.17. 10 ⁶ ± 0.6. 10 ^{5a,b}	1294	-	MS
Amines							
Dimethylamine	ammoniacal	8.29E. 10 ⁴ ± 0.4. 10 ⁴	nd	nd	< 600	-	MS
Others							
Thioaceticacid	nd	nd	nd	3.01E. 10 ⁶ ± 0.6. 10 ⁶	717	-	MS
trans-3-Methyl-4-octanolide (trans-Whiskeylactone)	nd	nd	1.08. 10 ⁵ ± 0.5. 10 ⁴	nd	827	-	MS
2H-Pyran, 2-ethenyltetrahydro-2,6,6-trimethyl-	nd	1.39. 10 ⁵ ± 0.5. 10 ^{5a,b}	nd	3.41. 10 ⁵ ± 0.5. 10 ^{5a}	979	960	MS+LRI
Secondary Volatile Compound (GC-FID)							
		Mean (mg.L-1) ± Standard deviation ⁵				MR	
		QM	SB				
Acetaldehyde	fruit	34.54 ± 8.50 ^b	122.05 ± 8.02 ^a	48.07 ± 9.87 ^b			
Ethyl acetate		nd	40.83 ± 11.87 ^a	47.72 ± 4.14 ^a			
Methanol		164.95 ± 11.60 ^a	141.48 ± 6.10 ^a	80.17 ± 10.41 ^b			
1-propanol	alcoholic, pungent	nd	29.89 ± 2.79 ^a	18.96 ± 1.31 ^b			
2-methyl-1-butanol±3-methyl-1-butanol	fruit	111.35 ± 2.84 ^a	80.35 ± 3.65 ^b	86.55 ± 7.05 ^b			

¹ Aroma description obtained from an online database available at The LRI & Odour Database (2017), Jewison et al. (2012) and Jiang et al. (2013); ² Peak area count values, Mean ± Standard Deviation of the triplicate injection of samples; ³ LRI (literature) from Jiang et al. (2013) and Weddegeris et al. (2011); ⁴ MS ± LRI, mass spectrum and LRI according to a compound run in the VF-5 MS column, and the MS mass spectrum according to the reference spectrum in the NIST/EPA/NIH database and LRI that agreed with the literature. nd: Compound not identified; ⁵ Mean ± Standard Deviation of secondary volatile compounds quantified using the straight-line equation obtained from the injection of a standard curve of compounds into a gas chromatographer with flame ionization detector. ^{a,b} Different letters in the column denote statistically difference between means from attributes (p < 0.05). QM (Isabella and Ives), SB (Isabella and Ives), MR (Isabella and Ives wine).

Esters. The esters propanoic acid, dimethyl ester, ethyl butyrate, ethyl hexanoate, diethyl succinate and ethyl octanoate were identified in all of the wines at high “peak area count” values. Butyric acid (2-methylbutyl ester) was only detected in the QM wine. These compounds contribute to a wine’s fruity aroma and result from the reaction of acetyl coenzyme A (acetyl-CoA) with higher alcohols generated from amino acid or carbohydrate degradation (Welke et al., 2012). Esters are associated with fruity descriptors: butyric acid, 2-methylbutyl ester and ethyl hexanoate with apple aroma, propanoic acid, dimethyl ester and ethyl butyrate with pineapple aroma, and ethyl succinate with apple and strawberry aromas (Jiang et al., 2013). Tao et al. (2008) detected isoamyl acetate, isoamyl lactate and octanoate acetate in red wine made from vinifera cultivars and found aroma notes related to banana, raspberry and fruity aroma, respectively, and these compounds had a positive contribution to wine quality. This study detected isoamyl acetate in all of the wines and isoamyl lactate in only the SB wine.

Terpenes. Terpene compounds can be found in their free or conjugated form in cultivars and are released by endogenous enzyme activity present in grape skin during the crushing process, hence their presence in grape must. Terpenes are the secondary plant constituents that are biologically synthesized by the cytosolic mevalonic acid pathway from three acetyl-CoA molecules or by the pyruvate and glyceraldehyde 3-phosphate pathway (Robinson et al., 2014). Eucalyptol, α -terpineol and α -terpinene, acetate were found in all three wines and contribute to the eucalyptus essential oil and floral aromas, respectively. The highest number of terpene compounds was observed in the QM wine, and α -terpinene, which is associated with the citrus, lemon-lime aroma, was only found in this wine. Weldegeris et al. (2011) reported that the terpene composition of wine varies according to production region, grape composition and variations in fermentation conditions. These authors also detected terpinolene, limonene, 4-terpineol acetate, o-cymene, eucalyptol, α -terpinene and α -terpineol.

Alcohols. Among the ten alcohols identified, the QM wine exhibited the greatest variability in compounds. Alcohols are compounds that are synthesized from the secondary products of yeast metabolism via two mechanisms. They can be made in yeast through the anaerobic pathway from glucose or the catabolic pathway from the corresponding amino acid (valine, leucine, phenylalanine and isoleucine), or they can be synthesized in cultivars during pre-fermentative processes including crushing, pressing and skin contact (Fariña et al., 2014). Only 1-propanol and phenylethyl alcohols were observed to have high “peak area counts” in the three wines. These two alcohols are associated with the alcoholic and the floral aroma, respectively. Another alcohol, 1-hexanol, was only detected in the SB wine and is associated with a wine’s herbal aroma (Rocha et al., 2004). In the QM and MR wines, linalool was detected, which contributes to the floral aroma (Jiang et al., 2013). It is of note that this volatile varied significantly among the analyzed samples, suggesting that although the wines were produced with the same cultivars, other production factors, such as the time of grape harvesting and the technological processes used, might influence the aroma of the final product.

Aldehydes and ketones. According to Weldegeris et al. (2011), aldehydes and ketones are highly volatile components in alcoholic beverages and result from the direct oxidation of the corresponding alcohols and fatty acids, respectively. In this study, only 5 compounds from these classes were identified, and they varied among the studied wines. The compound 3-heptene-2-one was only found in the QM wine and confers a fruity aroma. Ionone was detected in all of the analyzed wines and confers a rose oil, violet aroma.

Amines. Only one amine compound was detected in the QM wine – dimethylamine, which contributes to the ammoniacal aroma of wines. Manetta et al. (2016) detected an amine compound in wines and suggested it might have been influenced by the winemaking process. At high concentrations, amines may be responsible for undesirable toxicological effects as headache, respiratory distress, heart palpitation, hypotension, hypertension and nausea.

Other compounds. The presence of thioacetic acid was detected in the MR wine (Perestrelo et al., 2006). In addition, a lactone was observed in the SB wine, and a pyran compound was found in the QM and MR wines.

Previous studies have determined the importance of furaneol to Isabella wines, as it has been described as a strawberry-like aroma. Other volatile compounds such as: beta-damascenone, methyl anthranilate and 2-aminoacetophenone have been reported as important contribute to aroma in red wine; however, these compounds were not identified in our red wines (Nelson et al., 1977; Pinho and Bertrand, 1995).

Higher alcohols, acetaldehyde and ethyl acetate concentrations

Significant differences were observed in the acetaldehyde, methanol and higher alcohol concentrations between the red wines (Table 3). Analysis revealed that the acetaldehyde content, which can negatively affect the aroma quality of wines because of its association with microbial ethanol oxidation under aerobic conditions, was greater in the SB wine (122.05 mg L⁻¹) compared to the other two wines. Aldehydes affect the sensory characteristics of beverages and might be an indicator of deterioration (Azevêdo et al., 2007).

The ethyl acetate content, which contributes to a fruity aroma, was below the perception threshold of 180 mg L⁻¹ (Rizzon and Miele, 2004) in the red wines, with concentrations of 40.83 and 47.72 mg L⁻¹, and was absent in the QM wine. At low concentrations, this compound represents a good sanitary grape conditions and favorable alcoholic fermentation conditions because its formation is associated with yeast and, in particular, acetic bacteria metabolism. The methanol concentration in all of the wine samples was below the maximum recommended and legislated limit (maximum 300 mg L⁻¹, Brasil, 2004), which are concentrations that favor wine quality, considering that methanol is highly toxic. Methanol results from pectin hydrolysis and therefore is dependent on the maceration of the solid parts of the grape bunch, especially the skin. The 1-propanol content found in the wines varied from 18.96 mg L⁻¹ (MR) to 29.89 mg L⁻¹ (SB). The 2-methyl-1-butanol and 3-methyl-1-butanol alcohol levels were higher in the QM wine (111.35 mg L⁻¹), an amount that

can significantly influence the sensory properties of the wine, especially regarding the aroma and fruity descriptions correlation. The concentration of higher alcohols is related to fermentation conditions such as temperature, oxygenation, and nitrogen and SO_2 content. Similar results regarding methanol, acetaldehyde and higher alcohols were reported by Rizzon et al. (2000), who evaluated the agronomic and enological characteristics of the Isabella and Ives grape for producing common red wine.

3.3 Relationship between volatile compounds identified by SPME-CG-MS and aromatic compounds

To determine whether sensory differences resulting from grape composition and production methods are related to specific chemical compounds or groups of compounds, principal component analysis (PCA) was performed. To analyze the volatile compounds profile, the sum of the chemical classes was expressed as “peak area count” values. Overall, the PCA results suggested that the aroma descriptors and volatile compounds discriminated between the analyzed wines (Figure 1).

In the quantitative descriptive analysis of the PCA graph (Figure 1a), the aroma descriptors are indicated by vectors, and the wines are represented by squares. In this type of analysis, the higher the decomposition of the vector along the component axes, the greater its importance in evaluating the differences and similarities among the wines. It was observed that the Principal Component (PC1) accounted for 58% of the variability among wines, and some variability was explained by the Second Component (41%).

PCA showed that the wines exhibited different characteristics. The SB wine was mainly characterized by the descriptors tropical fruits and honey. The MR wine exhibited a greater floral, ripe fruit and pungent aroma intensity, and the QM wine, located on the right side of Figure 1a, was characterized by the majority of descriptors: spices, hot, volatile acidity, licorice, ripe grape and red fruits.

In Figure 1b, the chemical compound classes and higher alcohols, acetaldehyde, methanol and ethyl acetate are represented by vectors that characterize the wines close to them, and the

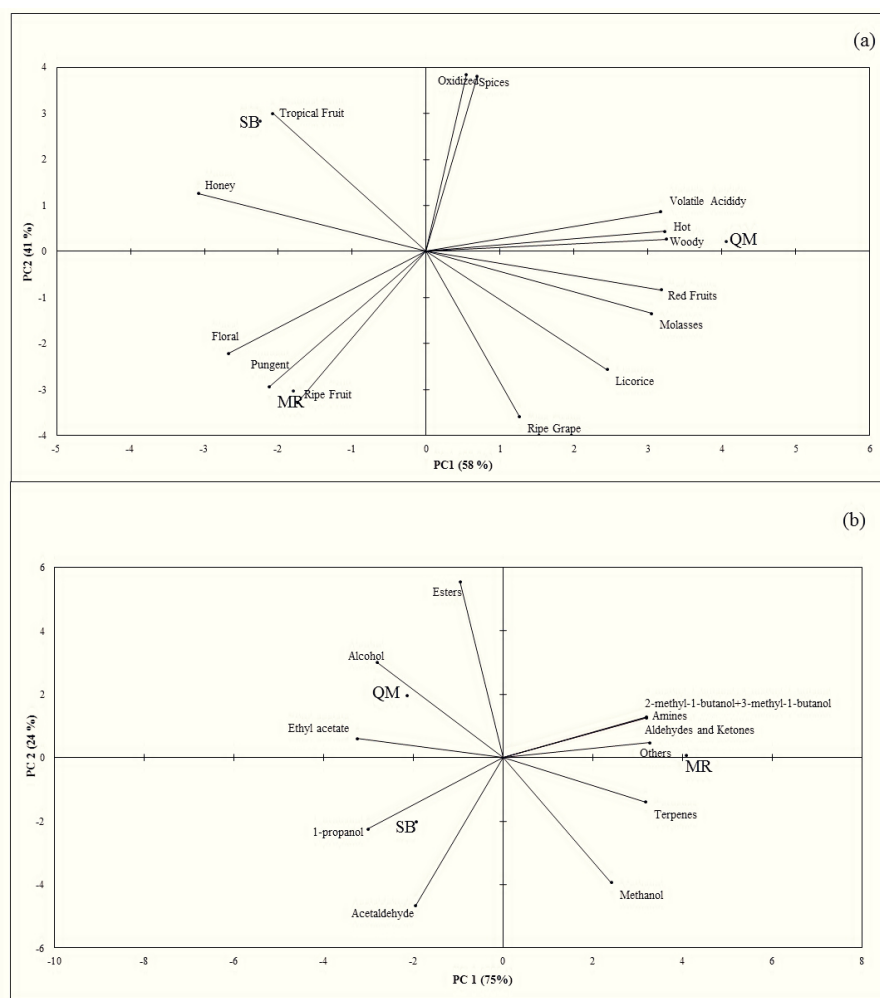


Figure 1. (a) Projection of aroma descriptors and samples of red wine produced with Isabella and Ives cultivars on the first two principal components and (b) projection of the higher alcohols, acetaldehyde, ethyl acetate and volatile compounds profile according to chemical classes and samples of red wine produced with Isabella and Ives cultivars on the first two principal components.

samples are represented by a square corresponding to the mean of the replicates. The wines have different profiles regarding the area of each chemical compound class and the concentration of acetaldehyde, ethyl acetate, higher alcohols and methanol. The MR wine was unique compared to the others, exhibiting a distinct profile. The SB and QM wines are characterized by the presence of acetaldehyde, 1-propanol, ethyl acetate, alcohol and esters classes of compounds, according to Axis I. This accounted for 75% of the variation among the wines, indicating that the more to the left the vectors are located, the greater the relative area of these compounds. The MR wine stood out from the other wines due to its higher area values for amines, others, aldehydes and ketones, terpenes, methanol and higher alcohols.

The differences uncovered with the PCA might have been influenced by the varietal composition of the wines. Furthermore, although the wines are originated in the same region, they are from different wineries that each have a unique production process.

These differences might also be attributed to the identified compounds. The alcohol and esters class might explain why the QM wine had aroma descriptors (pungent and tropical fruits) with significant difference, when compared to the other wines (Table 2). In general, Tecchio et al. (2007) reported that wines produced with *V. labrusca* cultivars were also characterized by a fruity aroma/flavor due to the presence of esters.

4 Conclusion

The aroma descriptors and volatile compounds confirmed essential information about the Isabella and Ives red wines. The results indicate that the grape composition of each wine mainly influences the sensory quality and the presence of volatile compounds in red wine. In addition to grape variety, the production process also contributes to the final sensory expression of the wine. According to PCA, fruity descriptors were the primary contributor to the aroma profile of the analyzed wines due to the presence of ethyl acetate and esters, especially in the wine coded as QM, which exhibited the highest variety of compounds. However, considering the continuous growth of Brazilian wine production and the importance of volatile compounds for their flavor, further characterization studies are essential to improve the method of producing such beverages.

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