AROMA PROFILE OF TEMPRANILLO TROPICAL RED WINES FROM DIFFERENT SEASONS IN THE SÃO FRANCISCO VALLEY, NORTHEAST OF BRAZIL

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Abstract
Aromatic characteristics of wines are strongly influenced by agronomical and enological factors, depending of the climate, cultivar and winemaking process. Tropical wines are a new concept of vitiviniculture that is being developed in the Northeast of Brazil since the 80's, located between 8-9º latitude of the South Hemisphere, where the second most important cultivar used for reds is Tempranillo. In this condition, vines produce grapes and enologists elaborate wines twice a year, because high temperatures, solar radiation and water availability for irrigation. The aim of this work was to determine aromatic profiles of Tempranillo red wines, from three vintages, by using gas chromatography-mass spectrometer (GC-MS). The volatile compounds were extracted by SPME and analyzed on a Shimadzu GC 2010 Plus instrument. The volatile compounds were identified by comparison of the mass spectra recorded by the spectrometer database-NIST, as well as matching the retention indices and mass spectra with the literature data. The concentration of the volatile compounds was determined and quantified by an external calibration curve. Results are discussed and showed that Tempranillo red wines presented different aroma profiles according to the vintage and seasons, and compounds were identified as esters, alcohols, acids, hydrocarbons and phenylpropanoid. The comparison of the results with data from the literature suggests that the Tempranillo tropical wines were influenced by climate and cultivar factors, presenting typicality that is highly valorized for wines from a determined region worldwide.
Keywords: Vitis vinifera L.; grape; Tempranillo; tropical wines; volatile compounds; typicality.

1 INTRODUCTION

Wine aroma is one of the most important characteristic that impacts on the quality of the beverage and is originated by the presence of several volatiles compounds (Jackson 2008; Ribéreau-Gayon et al. 2004). There are many factors influencing their presence and concentration in the wines, like the environmental conditions and viticultural practices (climate, soil, trellis and training systems, rootstock, cultivar, irrigation, nutrition) and the winemaking process (harvest date, fermentation temperatures, maceration time, aging, cork type) (Peynaud 1997; Usseglio-Tomasset 1995).

In Brazil, the production of wines using *Vitis vinifera* L. varieties is recent, even in the traditional regions of the country localized in the South, where the activity started there are 30 years ago. Additionally, Brazilian tropical wines had been made since the 80's, in the São Francisco river Valley, region localized in the Northeast of Brazil. It is a new winegrowing region, presenting a tropical semiarid climate with some peculiars characteristics allowing to scale grape harvests for winemaking throughout the year (Tonietto and Teixeira 2004), due to the high temperatures, sunlight and water availability for irrigation. Grapes and wines elaborated in different months of the same year present variations in composition, quality and typicality (Pereira *et al*. 2008, 2010 and 2011; Lima et al 2011). Thus, the aim of this work was to evaluate the physicochemical composition and the volatile profiles of three Tempranillo wines, elaborated with grapes harvested three times in 2009 and 2010.

2 MATERIAL AND MEHTODS

The climate of this region is a BSwh' by the Köppen classification and the majority of soils are podzols. The area presents a climate with intra-annual variability, with an annual average temperature of 26.5ºC (21.0ºC for minimum and 31.5ºC for maximum temperatures), and it is located at 350 m above of sea level, in a flat landscape (Tonietto and Teixeira 2004; Teixeira 2001; Teixeira and Azevedo 1996). The rainy season occurs from December to March, with about 567 mm of normal rainfall (Teixeira 2001). The heliotermical availability (about 3000 hours of luminosity.year^{-1}) allows a continuous vegetative development, and the grapevine cropping is possible throughout the year.

Tempranillo vines were planted in 2005, grafted onto '*IAC-766*' (*106-8* x *Vitis caribeae*). The vineyard is trained in vertical shoot positioning and pruning system is a unilateral spur pruned cordon. The density of plantation is 4,000 vines/ha (2.5 m between rows and 1 m in row) and row orientation is North-South.

Grapes were harvested in the morning in three different vintages, in June and November of 2009, and in November 2010, according to the evaluations of the alcohol degree potential, total acidity, and skin and seed tasting. Grapes were transported to the Laboratory of Enology at Embrapa Tropical Semi-Arid, in Petrolina-Pernambuco Estate, Brazil and left for twenty hours in cold chamber at 10ºC. Grapes were de-stemmed and lightly crushed, and sulfur dioxide was added (5 g.100 L^{-1}). The alcoholic fermentation was carried out in a stainless steel tank of 200 L, at 25±2ºC, with addition of commercial *Saccharomyces cerevisiae* (30 g.100 L^{-1}), during about five days of maceration. Then, wine was pressed and started the malolactic fermentation at 18±2ºC for twenty days. At the end, wines were stabilized at 0ºC for one month (Peynaud 1997; Boulton et al. 1996). Wines were bottled and analyzed one month later. The classical analysis were performed to determine the pH value, alcohol content, total and volatile acidity and the free sulphur dioxide content. The volatile compounds were identified by HS-SPME coupled with GC-I-MS and quantified using GC-FID (Canuti et al 2009; Adams 2007; Cullerè et al 2004; AOAC, 1998; OIV, 1990).

Extraction of the volatile compounds

The volatile compounds were extracted by SPME in headspace mode utilizing a 1 cm fused-silica fiber coated with 100 μm polydimethylsiloxane coating (Supelco, Bellefonte, USA). Eight milliliters of wine were placed in a 20 mL amber vial containing 3.0 g of sodium chloride, which was added to improve the extraction yield, and hermetically sealed. The samples were carefully stirred and heated at 40 °C for 15 min. Afterwards, the stirring was stopped, and the SPME fiber was introduced into the vial where it was maintained under heating for 30 min. The compounds were thermally desorbed in the GC injector port and analyzed. Before the analysis, the SPME fibers were conditioned according to the manufacturer's recommendations. The choice of the extraction conditions was based on previous works (Canuti et al 2009)

GC-MS Analysis

The GC-MS analysis was carried out on a Varian 450GC-240MS instrument (Kyoto, Japan) equipped with 30 m x 0.25 mm i.d. DB-Wax and VF-5MS capillary columns (1.0 μm film thickness), utilizing helium at 1.9 mL min^{-1} as carrier gas. The injections were at 240 ºC in splitless mode. The transfer line temperature and detector temperature were set at 240°C and 200°C, respectively. The oven temperature was maintained at 40 °C for 4 min, then it was raised to 80 ºC at 2.5°C min^{-1}. Afterwards, it increased from 80 to 110 °C at 5°C min^{-1} then to 220 °C at 10°C min^{-1} and was held there for 5 min (run time= 42 min). The retention indices were determined by the injection of a mixture of C_7-C_{30} homologous n-alkanes (Sigma, St. Louis, MO, USA) analyzed under the same chromatographic conditions of the wine samples. The mass spectra were obtained at 70 eV ionization energy in full scan mode. The identification of volatile compounds was performed through comparison of the mass spectra recorded with those provided by the spectrometer database (NIST 2.0 2008– 287,324 compounds), as well as matching the retention indices and mass spectra with the literature data using the same columns (Robinson et al 2011).

GC-FID Analysis

The GC-FID analysis was accomplished on a Shimadzu GC 2010 Plus instrument with quantitative purpose. The chromatographic conditions employed were similar to GC-MS analysis. However, nitrogen was used as carrier gas and make-up (30 mL/min). The major compounds identified in the wines were quantified by an external calibration curve in the concentration range from 0.7 to 300 mg L^{-1}. In order to attenuate the matrix effect, the stock solution was prepared as a 12% v/v hydroalcoholic solution (50 mL) with the pH adjusted to 3.4 and containing 6.0 g L^{-1} tartaric acid. The following analytical standards were used: 2-phenyl-etanol, isoamyl alcohol, 1-hexanol, hexanoic acid, octanoic acid, decanoic acid, ethyl lactate, diethyl succinate, ethyl decanoate, ethyl valerate, ethyl laurate, ethyl octanoate, and ethyl hexanoate.

3 RESULTS AND DISCUSSION

Table 1 shows the classical analyzes of Tempranillo wines. Physicochemical composition of the wines changed according to the season of the grape harvested, in the first or second semester of the year, as it was showed in previous works (Pereira et al. 2008). Normally, temperatures are lower in the first semester than those from the second semester (Tonietto and Teixeira 2004). Wines elaborated in June presented the lowest pH and alcohol content as compared to wines from November in both years (2009 and 2010). In addition, wines from November 2010 presented higher alcohol content than those from November 2009, because 2010 climate presented the highest

temperatures (data not showed). Results showed that antioxidant levels used in the winemaking were enough to protect wines, showing low volatile acidities.

Table 1: Classical analyzes determined in Tempranillo tropical wines from 2009 and 2010 vintages in the São Francisco Valley, Northeast of Brazil.

Parameters	Tempranillo wines		
	June/2009	November/2009	November/2010
pH	3.4^b	3.6^a	3.7^a
Alcool content (%v/v)	12.3^c	13.2^b	13.8^a
Total acidity (g L^{-1} tartaric acid)	6.8^a	5.5^b	5.3^b
Free SO_2 (mg L^{-1})	35.4^a	36.9^a	35.2^a
Volatile acidity (g L^{-1} acetic acid)	0.51^a	0.56^a	0.58^a

Means with same letter in the same line do not differ significantly by Tukey's test (p>0.05).

Twenty six volatile compounds were identified in the Tempranillo wines, but only twelve were quantified and are showed in the Table 2. Some compounds did not present variations in concentration according to the season, as isoamyl alcohol, ethyl lactate, 1-hexanol, ethyl hexanoate, ethyl octanoate and ethyl decanoate. While the others volatiles quantified in the Tempranillo wines were influenced by the environmental variations among the years 2009 and 2010 (2-pheylethanol, diethyl succinate, octanoic acid and decanoic acid) or between the season of the harvest of the year (ethyl laurate, hexanoic acid and decanoic acid). The 2-phenylethanol, a compound linked to the note of "roses" aroma in wines (Peinado et al. 2004), presented the highest values in the Tempranillo wines from November 2010 (35.32 mg L^{-1}) and June 2009 (33.80 mg L^{-1}), and the lowest in the wines from November 2010 (19.71 mg L^{-1}). As discussed above, climatic conditions of the second semester of 2010 presented higher temperatures than those in the second semester of 2009 season, and could promote the loss of floral aroma in wines from 2010.

Table 2: Concentrations (mg L^{-1}) of the major volatile compounds identified in Tempranillo tropical wines from 2009 and 2010 vintages in the São Francisco Valley, Northeast of Brazil.

Volatile compounds (mg. L^{-1})[1,2]					Tempranillo wines		
	KI*	KI*Lit.	KI#	KI#Lit.	June/2009	November/2009	November/2010
Isoamyl alcohol	754	740	1233	1230	38.33 ± 1.82^a	45.64 ± 6.92^a	37.17 ± 9.11^a
Ethyl lactate	825	815	1363	1363	1.75 ± 0.14^a	1.33 ± 0.26^a	1.86 ± 0.57^a
1-hexanol	886	870	1375	1360	3.07 ± 0.34^a	2.18 ± 0.29^a	2.66 ± 0.50^a
Ethyl hexanoate	1023	996	1223	1240	14.70 ± 1.03^a	13.16 ± 1.81^a	13.14 ± 1.81^a
2-phenylethanol	1130	1122	1937	1931	33.80 ± 0.65^a	35.32 ± 3.93^a	19.71 ± 2.74^b
Diethyl succinate	1192	1179	1690	1689	12.55 ± 0.33^b	12.06 ± 0.37^b	16.69 ± 1.73^a
Ethyl octanoate	1207	1197	1432	1436	0.52 ± 0.02^a	0.56 ± 0.04^a	0.49 ± 0.04^a
Ethyl decanoate	1398	1395	1642	1636	0.14 ± 0.01^a	0.18 ± 0.01^a	0.11 ± 0.05^a
Ethyl laurate	1594	1595	1845	1849	0.05 ± 0.00^b	0.06 ± 0.00^a	0.05 ± 0.00^{ab}
Hexanoic acid	970	973	1869	1872	4.43 ± 0.04^a	3.87 ± 0.13^b	3.86 ± 0.18^b
Octanoic acid	1195	1171	2091	2083	19.71 ± 0.75^a	20.98 ± 0.54^a	17.24 ± 0.10^b
Decanoic acid	1374	1366	2307	2307	29.01 ± 1.72^b	32.81 ± 0.17^a	18.22 ± 1.92^c
Total compounds					158.06	168.15	131.20

[1]Compounds identified by comparing their mass spectra with mass spectra library NIST and Kovats retention indices (KI) with the literature. *KI on a DB-5MS column or similar. #KI on a DB-Wax column or similar. [2]Means with same letter on the same line do not differ significantly by Tukey's test (p>0.05).

On the other hand, diethyl succinate was higher in the wines from November 2010 than the others, and this compound is linked to the esters degradation, and a synthesis of new esters occurs, being described as "vinous aroma" (Welke et al 2013; Usseglio-Tomassetti 1995). This result highlight the possibility of wines from November 2010 had a strong influence of high temperatures and more risk of degradation. Welke et al (2013) identified diethyl succinate as marker of Sauvignon blanc wines elaborated with grapes from the South of Brazil, and also in Chardonnay wines (1.68 mg L^{-1}). In Tempranillo wines in this paper the concentration found varied between 12.06 (November 2009) to 16.69 mg L^{-1} (November 2010).

The compounds hexanoic, octanoic and decanoic acids, linked to the "fatty" and "cheese" aromas (Peinado et al. 2004). Hexanoic acid was high in Tempranillo wines from June 2009 (4.43 mg L^{-1}), while octanoic acid was high in wines from November 2009 (20.98 mg L^{-1}) and June (19.71 mg L^{-1}). Welke et al (2013) found in Chardonnay wines from the South of Brazil 0.04959 mg L^{-1} of hexanoic acid, 160.320 mg L^{-1} of octanoic acids and 0.180 mg L^{-1} of decanoic acid. The difference can be explained by the cultivar and also the different climatic conditions of the regions studied (Ribéreau-Gayon et al. 2004).

4 CONCLUSLION

In this work, different chemical characteristics of Tempranillo wines were showed, according to three different seasons evaluated, where grapes were harvested in one season of the first and two seasons of the second semester.

Wines from June presented the lowest pH and alcohol content as compared to wines from November in both years, while wines from November 2010 presented higher alcohol content, because 2010 climate presented the highest temperatures.

High concentration of diethyl succinate was determined in wines from November 2010, the warmest season, showing that this compound is confirmed to be a marker of wine oxydation. The highest concentrations of 2-phenyelethanol were determined in the wines from June and November 2009.

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