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Use of solid phase microextraction to identify volatile organic compounds in brazilian wines from different grape varieties

Identificação de compostos orgânicos voláteis de vinhos brasileiros de diferentes castas por microextração em fase sólida

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Abstract

The Brazilian wine industry has shown significant growth in recent years and the insertion of new concepts, such as geographical indications as signs of quality, has placed Brazil in tune with the tendencies of world wine production. The aim of this work was to apply the Solid Phase Microextraction technique in combination with Gas Chromatography-Mass Spectrometry to study Brazilian wines made from different grape varieties, in order to separate and identify their volatile organic compounds. These substances were identified by comparisons between the spectra obtained with those presented in the NIST library database, and by comparisons with linear retention indices and literature data. The amounts of the compounds were calculated based on the total peak areas of the chromatograms. Forty-seven volatile compounds were identified and grouped into alcohols, aldehydes, fatty acids, esters, hydrocarbons, ketones and terpenes. Most of them belonged to the ester function, conferring a fruity aroma on the wines. The alcohols may have originated from the yeast metabolism, contributing to the alcoholic and floral aromas. Ethyl lactate, 1-hexanol and diethyl maleate were identified in all the varieties, except Merlot. Decanal, methyl citronellate, (E)-2-hexenyl-3-methylbutyrate were only found in Merlot, while 2,3-butanediol was only present in the Tannat wines. 2-Phenylethanol was present in all varieties and is recognized as giving pleasant rose and honey attributes to wines. This study showed that the volatile profile of red wines is mainly characterized by esters and higher alcohols. The statistical analysis of the comparison of averages showed a greater amount of averages significantly different in the relative areas of Merlot wine. The Principal Component Analysis showed one grouping composed only of the Merlot wine samples, and this was probably related to the existence of the volatile organic compounds that were specifically identified in these wines.

Keywords: *Brazilian wines; Chemical compounds; SPME; GC-MS; Volatile profile; Red wines.*

Resumo

A indústria vinícola brasileira apresentou crescimento significativo nos últimos anos e a inserção de conceitos – como, por exemplo, indicações geográficas como sinais de qualidade – colocou o Brasil em sintonia com as tendências da produção mundial. Este trabalho objetivou aplicar a técnica de microextração em fase sólida em combinação com a cromatografia gasosa-espectrometria de massa, para analisar vinhos brasileiros produzidos com diferentes variedades de uva, a fim de separar e identificar seus compostos orgânicos voláteis. Estes foram identificados por meio de comparações entre os espectros obtidos e os da base de dados da biblioteca do *National Institute of Standards and Technology* (NIST), e por comparações com índices de retenção linear e dados da literatura. A quantidade de compostos baseou-se nas áreas de pico total de cromatogramas. Quarenta e sete compostos voláteis foram identificados e agrupados em álcoois, aldeídos,

ácidos graxos, ésteres, hidrocarbonetos, cetonas e terpenos. A maioria pertencia à função éster, conferindo aroma frutado aos vinhos. Os álcoois podem ter se originado a partir do metabolismo da levedura, contribuindo para aromas alcoólicos e florais. Lactato de etila, 1-hexanol e maleato de dietila foram identificados em todas as variedades de vinho, exceto Merlot. Decanal, citronelato de metila, 3-metilbutirato de (E)-2-hexenila foram encontrados apenas em Merlot, ao passo que 2,3-butanodiol, somente em vinhos Tannat. O composto 2-Feniletanol foi encontrado em todas as amostras e é reconhecido por conferir agradáveis aromas de rosa e mel aos vinhos. Este estudo evidenciou que o perfil volátil dos vinhos tintos é caracterizado, principalmente, por ésteres e álcoois superiores. Análises estatísticas de comparação de médias mostraram uma maior quantidade de médias significativamente distintas das áreas relativas para o vinho Merlot. A análise de componentes principais mostrou que um grupamento foi formado apenas por amostras de vinho Merlot, o que está provavelmente relacionado à existência de compostos orgânicos voláteis que foram, especificamente, identificados nestes vinhos.

Palavras-chave: *Vinhos brasileiros; Compostos químicos; SPME; GC-MS; Perfil de compostos voláteis; Vinhos tintos.*

1 Introduction

Wine is an alcoholic beverage with a considerable world production rate (approximately 2.7×10^{10} L per year), and wine production has increased in Brazil in recent years from 2.6×10^{8} L in 2012 (ASSIS et al., 2014) to 4.4×10^{8} L in 2015 (IBRAVIN, 2016). In Brazil, the wine market presents a complex behaviour because each producing region has different characteristics, such as climate, soil types and topography that influence the final quality of the beverage (EMBRAPA, 2016).

The main states producing fine wines are Rio Grande do Sul, Santa Catarina and the São Francisco Valley (states of Pernambuco and Bahia), Rio Grande do Sul being responsible for more than 90% of the total Brazilian production. In these regions, American vines predominate since they have adapted to the climatic conditions, especially to the rainy season, which coincides with grape ripening. Technological and genetic modernization has also favoured Brazilian participation in the international market of table grapes, juice and wine (MIELE; RIZZON, 2011)

Brazilian legislation defines wine as a drink produced exclusively from the alcoholic fermentation of fresh, ripe grapes or from fresh grape juice (BRASIL, 1988). Wine is an extremely complex product and has a great diversity of aromatic compounds. Its flavour is a mixture of volatile and non-volatile components. Volatile compounds contribute significantly to the sensory properties of many beverages, including wines. These compounds are mainly influenced by the grape variety, and some of them are formed during the winemaking process, especially during the fermentation and aging steps. The determination of the amounts of each volatile component can be used to establish beverage quality and authenticity, in order to improve wine quality (NICOLLI et al., 2015).

Various techniques, such as liquid-liquid extraction (LLE), solid phase extraction (SPE), dispersive liquid-liquid microextraction (DLLME), stir bar sorptive extraction (SBSE) and solid phase microextraction (SPME) have been employed for the characterization of volatile wine components (RAMOS, 2012). Solid-Phase Microextraction (SPME) in combination with gas Chromatography–Mass Spectrometry (GC–MS) has been implemented as the method of choice for the analysis of volatile patterns in foods, including wines (SIQUEIRA; BOLINI; MACEDO, 2008). It has been widely used for the analysis of aroma volatiles in many food and beverage matrices. This technique eliminates the use of organic solvents that can be toxic, and constitutes a methodology that makes use of the Green Chemistry principles (CHEVANCE et al., 2002). Moreover, it allows more samples to be processed, generating large amounts of data that demand new interpretation procedures. Chemometric tools such as the Principal Components Analysis (PCA) are also required to use this information in a comprehensive way.

Despite the socio economic importance of wine production in Brazil, few studies have been carried out to determine their volatile compounds. Considering the need to characterize these beverages, the aim of this study was to identify the volatile components of wines made with distinct grape varieties (Cabernet Sauvignon, Merlot, Cabernet Franc, Pinot Noir and Tannat) from different Brazilian regions, using SPME and GC to assess their volatile organic profiles. The present work also intends to provide new perspectives for the discovery of a potential chemical signature for these products that might be used to assess their quality.

2 Materials and methods

2.1 Samples

The red wines analysed in this study were produced in different regions of Brazil, namely the states of Minas Gerais, Rio Grande do Sul and the São Francisco Valley (states of Pernambuco and Bahia) (Table 1). Wine samples from five different grape varieties cultivated in Brazil were obtained from the Belo Horizonte market, eight being from Merlot grapes (alcohol content ranging from 11.0 to 13.0%); nine from Cabernet Sauvignon (alcohol content ranging from 10.5 to 13.0%); five from Cabernet Franc (alcohol content

Table 1. Sample codes, grape varieties, region and year of production and alcohol content of the Brazilian red wines analysed in this study.

Sample codes[a]	Grape variety	Production region[b]	Alcohol content (%)
1	Merlot	MG	12.5
2		RS	12.0
3		RS	11.0
4		RS	13.0
5		MG	13.0
6		RS	12.5
7		RS	11.8
8		RS	12.0
9	Cabernet Sauvignon	RS	12.0
10		RS	13.0
11		MG	12.0
12		RS	12.0
13		MG	13.0
14		RS	13.0
15		RS	11.5
27		SFV	10.5
28		SFV	13.0
16	Cabernet Franc	RS	11.0
17		RS	11.5
18		RS	11.5
29		MG	13.7
30		RS	13.0
19	Pinot Noir	MG	13.0
20		MG	12.0
21		RS	12.0
22	Tannat	MG	12.0
23		RS	13.0
24		MG	12.5
25		RS	11.5
26		MG	13.5

[a]All red Brazilian wine samples were produced in 2013; [b]MG: wines produced in Minas Gerais state; RS: wines produced in Rio Grande do Sul state; SFV: wines produced in the São Francisco Valley (states of Pernambuco and Bahia).

ranging from 11.0 to 13.7%), three from Pinot Noir (alcohol content ranging from 12.0 to 13.0%) and five from Tannat (alcohol content ranging from 11.5 to 13.5%).

2.2 Extraction conditions

Ten millilitres of each sample were placed in a 20-mL vial containing 3.0 g of NaCl. Prior to extraction, the solution was hermetically closed with a Teflon septum and aluminium cap, and heated at 55 °C for 10 min with constant stirring (500 rpm). The SPME device was then inserted into the sealed vial by manually penetrating the septum, the fibre exposed to the headspace, and the extraction carried out at 55 °C for 30 min. This procedure was adapted from Arcanjo et al. (2015) and Barros et al. (2012). After extraction, the needle on the SPME manual holder was inserted into the GC injector, and the fibre directly exposed to the hot injector port at 250 °C in the splitless mode for 10 min. The analyses were carried out using divinylbenzene-carboxen-polydimethylsiloxane (DVB-CAR-PDMS) fibres with a manual holder (Supelco, Bellefonte, PA, USA).

2.3 Gas chromatography–mass spectrometry conditions

A Hewlett Packard model 6890N Series II gas chromatograph annexed to an HP 5975C mass spectrometer was used together with an inert MSD Triple-Axis Detector. The analytical column was an HP-5MS (30 m × 0.25 mm i.d. × 0.25 µm film thickness). The carrier gas was helium at 1.3 mL/min. The temperature program started at 40 °C for 2 min, and then increased to 250 °C at 10 °C/min, where it was held for 2 min. The spectrometric conditions were electronic impact (ionization energy, 70 eV) and a source temperature of 250 °C. The retention data from a series of n-alkanes (C8–C20) employed under the same experimental conditions for the chromatographic analysis of volatile compounds in wine was used to calculate the experimental linear retention indices. The mass spectrometric information from each chromatographic peak was compared with data in the NIST mass spectra library. Each analysis was carried out in triplicate and the abundance of a particular compound was quantified using its peak area. The compounds were tentatively identified by comparisons with data in the literature, mass spectral data, retention times and linear retention indices (ADAMS, 2007). The amount of each compound was calculated based on the total peak area (or the sum of individual peak areas) from the GC-MS chromatograms.

2.4 Statistical analysis

Tukey's test and the Games-Howell's test were applied at 5% probability ($p < 0.05$) with a 95% confidence level to analyse the differences between the means of the volatile compounds. The areas of the volatile components identified in the samples were used in the Principal Component Analysis (PCA) to verify similarities by grouping. A covariance matrix of 70% was used as the minimum acceptance threshold for the cumulative proportion of explained variance, and the Minitab: 18.1 to process the data (MINITAB INC, 2017).

3 Results and discussion

Table 2 shows the volatile organic profile of the Brazilian red wines analysed in this study. The organic volatile compounds were grouped into different chemical classes. Forty-seven volatile compounds were identified

Table 2. Relative areas of the volatile compounds characterized in the Brazilian wine samples by SPME and GC-MS.

Compounds	LRI[b]	Percentage of relative area (mean ± standard deviation) of the volatile compounds in the wine samples[a]				
		Merlot	Cabernet Sauvignon	Cabernet Franc	Pinot Noir	Tannat
Alcohols						
ethanol	460	37.09 ± 6.10[a]	40.97 ± 8.81[a]	41.77 ± 8.78[a]	34.66 ± 2.50[a]	45.28 ± 8.94[a]
3-methyl-1-butanol	742	15.14 ± 1.96[a]	16.65 ± 2.36[a]	16.73 ± 2.93[a]	12.74 ± 2.11[a]	14.51 ± 1.63[a]
2,3-butanediol	789	-	-	-	-	0.63 ± 0.08
1,3-butanediol	791	-	0.11 ± 0.25	-	-	-
1-hexanol	864	-	0.15 ± 0.16[a]	0.74 ± 1.17[a]	0.07 ± 0.01[a]	1.03 ± 2.05[a]
2-ethyl-1-hexanol	983	0.06 ± 0.18	-	-	-	-
1-methyl-1-heptanol	992	-	0.09 ± 0.10[a]	1.32 ± 2.72[a]	-	-
benzyl alcohol	998	0.07 ± 0.16[a]	-	-	0.01 ± 0.02[a]	0.01 ± 0.02[a]
2-phenylethanol	1043	10.25 ± 1.47[a]	10.03 ± 3.36[a]	9.53 ± 2.87[a]	6.10 ± 1.40[a]	7.28 ± 2.90[a]
1-octanol	1001	0.08 ± 0.08[a]	0.01 ± 0.03[a]	-	-	-
6-methyl-1-heptanol	1004	-	-	-	0.02 ± 0.04[a]	0.07 ± 0.02[a]
3,4-dimethyl cyclohexanol	1126	-	0.01 ± 0.02[a]	0.00 ± 0.02[a]	-	0.06 ± 0.01[b]
1-dodecanol	1298	0.21 ± 0.20[a]	0.29 ± 0.36[a]	0.25 ± 0.23[a]	0.26 ± 0.29[a]	-
Aldehydes						
(E)-2-nonenal	1160	0.06 ± 0.04		-	-	-
decanal	1205	0.22 ± 0.13[a]	0.01 ± 0.01[b]	-	-	-
Carboxylic acids						
hexanoic acid	959	-	0.68 ± 0.36	-	-	-
hexadienoic acid	1038	-	0.31 ± 0.12[a]	0.97 ± 0.38[a]	-	-
octanoic acid	1174		0.17 ± 0.09[a]	0.20 ± 0.10[a]	-	-
nonanoic acid	1254	0.14 ± 0.07[a]	0.07 ± 0.08[a]	0.73 ± 0.32[a]	-	-
Esters						
ethyl lactate	801	-	1.80 ± 0.53[a]	0.59 ± 0.20[a]	0.07 ± 0.01[a]	0.92 ± 0.36[a]
ethyl isovalerate	851	0.05 ± 0.03[a]	0.05 ± 0.04[a]	0.06 ± 0.03[a]	0.07 ± 0.00[a]	0.07 ± 0.01[a]
isoamyl acetate	875	1.22 ± 1.11[a]	1.22 ± 0.63[a]	1.47 ± 0.83[a]	1.78 ± 0.49[a]	1.04 ± 0.14[a]
ethyl hexanoate	989	1.46 ± 1.25[b]	1.75 ± 0.44[ab]	2.78 ± 0.70[ab]	3.53 ± 0.29[ab]	3.46 ± 0.71[a]
methyl 6-methyl heptanoate	1060	0.12 ± 0.01[a]	-	-	0.02 ± 0.01[a]	-
monoethyl succinate	1080	4.04 ± 2.00[a]	0.50 ± 0.28[b]	-	-	-
methyl 2-methyl octanoate	1150	2.56 ± 0.83	-	-	-	-
diethyl maleate	1154	-	3.83 ± 2.34[a]	3.43 ± 1.27[a]	4.47 ± 2.35[a]	3.32 ± 1.22[a]
phenylethyl formate	1176	0.62 ± 0.46[a]	0.42 ± 0.25[a]	0.55 ± 0.25[a]	0.69 ± 0.23[a]	0.32 ± 0.19[a]
methyl salicylate	1196	0.04 ± 0.01[a]	0.03 ± 0.02[a]	0.04 ± 0.00[a]	0.03 ± 0.03[a]	0.05 ± 0.01[a]
ethyl octanoate	1201	11.20 ± 3.90[a]	8.29 ± 4.20[a]	9.10 ± 3.57[a]	13.25 ± 2.64[a]	8.30 ± 4.22[a]
isopropyl octanoate	1209	0.06 ± 0.00[a]	0.04 ± 0.02[a]	0.03 ± 0.02[a]	0.03 ± 0.03[a]	0.06 ± 0.01[a]
(E)-2-hexenyl-3-methylbutyrate	1220	0.06 ± 0.01	-	-	-	-
heptyl isobutyrate	1231	0.39 ± 0.15[a]	0.31 ± 0.12[a]	0.37 ± 0.14[a]	-	0.17 ± 0.12[a]
methyl citronellate	1237	0.35 ± 0.28[a]	0.05 ± 0.14[b]	-	-	-
ethyl phenyl acetate	1239	0.15 ± 0.11[a]	0.22 ± 0.13[a]	0.24 ± 0.12[a]	-	-
ethyl nonanoate	1245	6.81 ± 2.07[a]	4.30 ± 3.04[a]	4.41 ± 1.68[a]	8.45 ± 2.64[a]	4.74 ± 2.56[a]
ethyl isopentyl succinate	1368	0.60 ± 0.16[a]	0.65 ± 0.33[a]	0.31 ± 0.42[a]	0.34 ± 0.06[a]	0.36 ± 0.14[a]
ethyl decanoate	1381	0.59 ± 0.17[a]	0.31 ± 0.20[b]	0.56 ± 0.53[ab]	0.59 ± 0.31[ab]	0.26 ± 0.18[ab]
3-hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate	1387	1.35 ± 0.28[a]	1.03 ± 0.62[a]	0.81 ± 0.71[ab]	1.30 ± 0.62[ab]	0.27 ± 0.18[b]
ethyl tridecanoate	1653	0.18 ± 0.02[a]	0.13 ± 0.01[a]	0.17 ± 0.09[a]	0.21 ± 0.12[a]	-

[a]Averages characterized with the same lowercase letter, in the same row, did not differ among themselves ($p < 0.05$) by the Tukey and Games-Howell tests; [b]LRI calculated linear retention index.

Table 2. Continued...

Compounds	LRI[b]	Percentage of relative area (mean ± standard deviation) of the volatile compounds in the wine samples[a]				
		Merlot	Cabernet Sauvignon	Cabernet Franc	Pinot Noir	Tannat
Hydrocarbons						
2,6-dimethylphenol	1152	0.09 ± 0.01	-	-	-	-
4-ethylphenol	1181	0.16 ± 0.02[a]	0.04 ± 0.02[a]	-	0.18 ± 0.03[a]	0.26 ± 0.05[a]
Ketones						
1,1-dimethoxy-2-propanone	936	0.59 ± 0.14[a]	-	-	0.02 ± 0.01[a]	0.17 ± 0.03[a]
Terpenes						
3-carene	1003	-	0.01 ± 0.00	-	-	-
5-caranol	1128	0.04 ± 0.02[a]	0.03 ± 0.01[a]	0.02 ± 0.01[a]	0.03 ± 0.01[a]	0.06 ± 0.01[a]
(-)-menthol	1169	0.03 ± 0.01[a]	0.02 ± 0.01[a]	0.03 ± 0.01[a]	0.03 ± 0.01[a]	-
lavandulol	1184	0.02 ± 0.01[a]	-	0.02 ± 0.01[a]	-	-

[a]Averages characterized with the same lowercase letter, in the same row, did not differ among themselves ($p < 0.05$) by the Tukey and Games-Howell tests; [b]LRI calculated linear retention index.

and grouped into alcohols (13), aldehydes (02), fatty acids (04), esters (21), hydrocarbons (02), ketones (01) and terpenes (04).

Some compounds were found only in wines produced with a specific grape variety. Ethyl lactate, 1-hexanol, diethyl maleate were identified in all wine grape varieties, except for Merlot. On the other hand, decanal, methyl citronellate and (E)-2-hexenyl 3-methylbutyrate were only identified in Merlot wine samples. 1-Methyl-1-heptanol was found in six wine samples of Cabernet Sauvignon and in all samples of Cabernet Franc wines. The volatile compounds 3,4-dimethyl cyclohexane, 2,3-butanediol, 6-methyl-1-heptanol were identified in all samples of Tannat wines and hexadienoic acid was found in all Cabernet Franc wines.

The chemical composition of wines can vary due to a wide range of factors but is caused primarily by the variety of the grapes, their growing conditions such as climate and soil, and the viticultural practices of the winemaker. In addition, the yeast strain, fermentation and the aging processes may also affect the taste and quality of the wines. The possibility of discovering a potential chemical signature for these products is of great interest to the Brazilian industry, as it could be an alternative to access the quality of this beverage. The identification of volatile compounds could also help in the process of indicating the geographical origin of the wines, increasing their value on the domestic and international markets. In addition, the characterization of the volatile compounds in Brazilian wines is a gain in scientific and technological information.

In the present study, 2,3-butanediol was only identified in the Tannat samples. This compound, together with its precursor, acetoin, are desirable substances frequently found in wines. The route to produce acetoin passes through diacetyl (2,3-butanedione) and 2,3-butanediol, which are important components in many product aromas. 2,3-Butanediol can be found in low concentrations in wines and does not generally affect the sensory quality of alcoholic beverages, but in high concentrations, it can modify the bouquet and body of the wine due to its slightly bitter taste and high viscosity. This compound is not degraded by the bacteria commonly present in wines (RIZZON; ZANUZ; MIELE, 1997).

The alcohols identified in the wine samples may have originated from the synthesis of secondary products during the yeast metabolism. This class of compounds most likely positively contributed to the alcoholic (3-methyl-1-butanol) and floral (2-phenylethanol) aromas of red wines made from Isabel grapes. These compounds can be recognized by their strong and pungent smell and taste and are related to herbaceous notes. At low concentrations, 1-Hexanol, for example, can contribute to the smell of grass and green leaves found in some wines (FARIÑA et al., 2014; JIANG et al., 2013)

In the present study, 2-phenylethanol was present in all the wine samples analysed and this compound is known to give pleasant rose and honey attributes to beverages (ALVES; NASCIMENTO; NOGUEIRA, 2005). It is a post fermentative aroma and is formed via the decarboxylation and reductive deamination of phenylalanine, or, more probably, by a mechanism analogous to the formation of other higher alcohols, namely, the decarboxylation of phenylpyruvic acid and its reduction to 2-phenylethanol (RANKINE; POCOCK, 1969). Alcohols, under appropriate conditions, play an important role in some aromatic wines, whereas in excess, they may confer pungent and unpleasant notes.

Ethyl lactate significantly increases after malolactic fermentation and is typical of aged wines (DAVIS et al., 1985; ANTALICK; PERELLO; REVEL, 2012). Of the volatiles quantified in the Vernaccia di Serrapetrona wines, those

contributing to the flavour, on the bases of the odour thresholds previously reported in the literature, are: 2-phenylethanol (with the attribute of rose and honey), isoamyl acetate (banana), ethyl hexanoate (fruity), ethyl octanoate (anise), and ethyl decanoate (fruity, fatty). All of the attributes afforded by these compounds are recognized as positive in wines.

Most of the compounds identified belong to the ester function, indicating that these wines have a fruity aroma. Fatty acids are formed during the first two phases of alcoholic fermentation, although they can be found at very low concentrations in the must before fermentation. Medium-chain fatty acids (C6 to C12) are produced primarily by yeasts as intermediates in the biosynthesis of long-chain fatty acids, and are prematurely released from the fatty acid synthase (FAS) complex. These compounds can directly contribute to the flavour of wine, or serve as substrates that participate in the formation of ethyl acetates. The C6, C8 and C10 medium-chain fatty acid ethyl esters are important flavour-active metabolites that are responsible for the highly desired fruit aroma character in wine (Duan et al., 2015). Fatty acids have also been described with fruity, cheesey, fatty and rancid notes. In the present work, d hexanoic, octanoic and nonanoic acids were identified, and also the esters ethyl hexanoate, octanoate, nonanoate and decanoate. Although the presence of fatty acids can be related to the appearance of negative odours, they are very important for the aromatic equilibrium in wines because they are opposed to the hydrolysis of the corresponding esters. Long chain fatty acids, such as decanoic, dodecanoic, and tetradecanoic acids have a reduced effect on the flavour of wines, however, the C6-C10 fatty acids have a positive effect on the global aroma quality.

In the present study, an attempt was made to verify the existence of distinct profiles amongst the wines by comparing the averages of the relative areas of the volatile compounds (Table 2). Considering the most abundant compounds, such as ethanol, 2-phenylethanol, 3-methyl-1-butanol acetate, ethyl nonanoate and ethyl octanoate, it can be seen that the averages of the relative areas did not show significant differences ($p < 0.05$). The same was true for most comparisons of the mean values of the least abundant volatiles (Table 2) although some patterns could be verified. For instance, some wines were characterized by specific volatile compounds such as Merlot (2-ethyl-1-hexanol, (E)-2-nonenal, (E)-2-hexenyl 3-methylbutyrate, methyl 2-methyl octanoate and 2,6-dimethylphenol), Cabernet Sauvignon (1,3-butanediol, hexanoic acid and 3-carene) and Tannat (2,3-butanediol).

Another interesting characteristic is the significant difference ($p <0.05$) in the mean values of the relative areas of the Merlot wine samples when compared to the average of the Cabernet Sauvignon (monoethyl succinate, methyl decanoate and decanal) and Tannat wines (ethyl hexanoate and 3-hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate). Thus, given the largest group of specific compounds found in Merlot wines and the significant differences reported, it is possible to estimate a peculiar volatile profile for the wines of this grape variety.

Cabernet wines showed specific volatile compounds, such as 1-methyl-1-heptanol, hexadienoic acid and octanoic acid, whose average relative areas did not present significant differences ($p < 0.05$). In addition, these wines presented a greater abundance of carboxylic acids and the absence of benzyl alcohol and 1,1-diethoxy-2-propanone in their constitution, which may indicate a potential similarity pattern. Besides, some volatile compounds were not detected in the following wines: Merlot (ethyl lactate, ethyl maleate), Cabernet Franc (4-ethylphenol), Pinnot Noir (heptyl isobutyrate) and Tannat (1-dodecanol, ethyl tridecanoate and (-)-menthol). In order to complement this comparative study, the Principal Component Analysis (PCA) was applied to verify possible groupings that could characterize different wine profiles.

The three principal components (PC1, PC2 and PC3) accounted for 86.2% of the total variance and provided discriminatory information for the wine samples, as can be seen in the score plots between PC1 × PC3 and PC2 × PC3 (Figure 1). In order to carry out this analysis, the relative areas of the volatile compound peaks were used and only the wine samples with a total area of non-identified compounds less than or equal to 6% were considered.

The results showed that the Merlot wine samples were grouped together but that the other varieties did not present this behaviour. The Merlot wine group was characterized by having samples with scores higher than 0.01 in PC3. None of the other samples formed groups containing only one type of grape variety. One hypothesis for this group separation could be the existence of volatile organic compounds that, in the present work, were specifically identified in Merlot wines.

When evaluating the coefficients of the loading plots (Figure 2), it was verified that the Merlot wine samples were grouped in PC1 × PC3 and PC2 × PC3 due to the greater abundance of the compounds monoethyl succinate and methyl 2-methyl octanoate, and the low contents of ethyl hexanoate and diethyl maleate. In addition, most Merlot samples presented high PC1 scores due to low ethanol contents and high ethyl octanoate and ethyl nonanoate levels.

In relation to the other wine types, it was observed that some samples of Cabernet Franc, Cabernet Sauvignon and Tannat were characterized by low values of PC1 and PC3, since they presented high levels of ethanol and low levels or the absence of monoethyl succinate, methyl 2-methyl octanoate, ethyl octanoate and ethyl nonanoate. The other wines presented varied ethanol

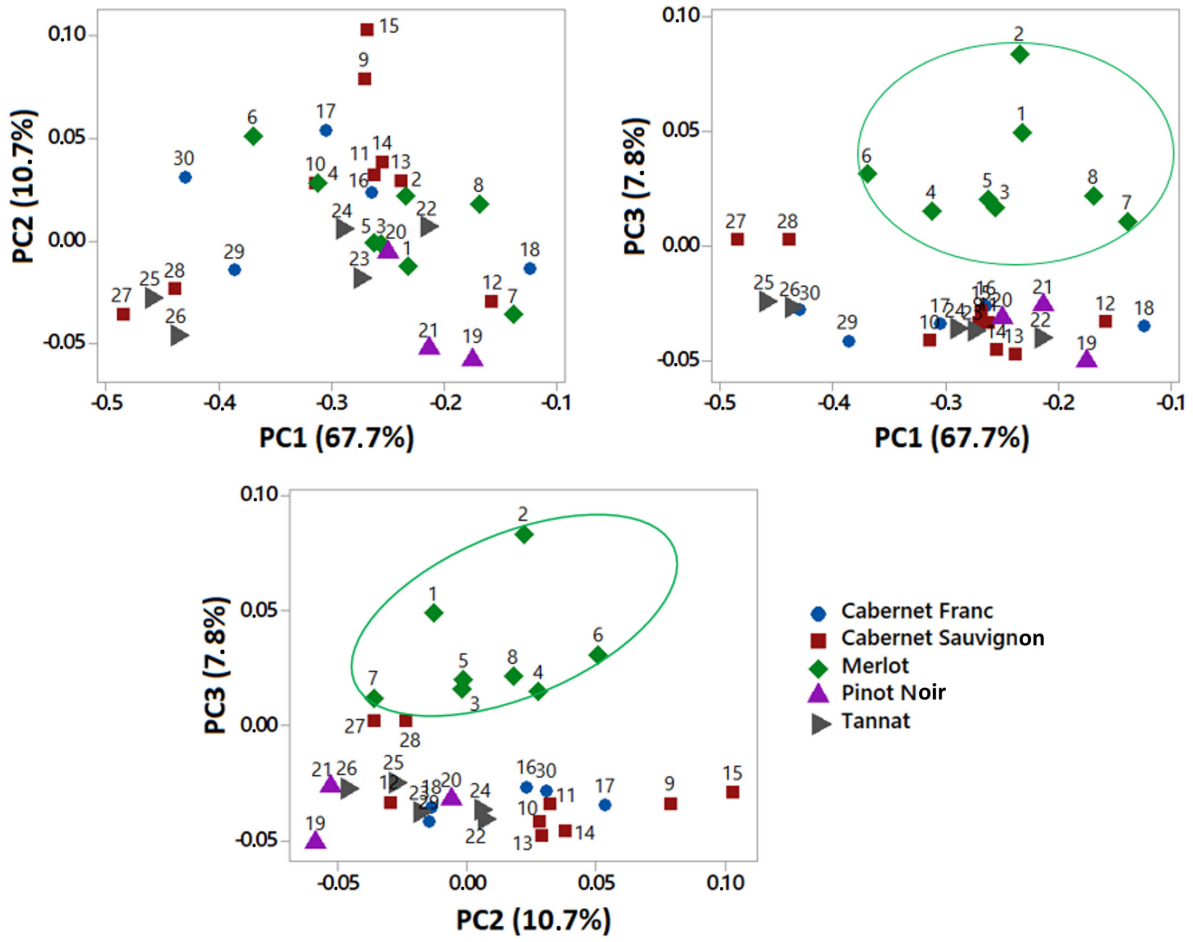

Figure 1. PCA score plots (PC1 × PC2, PC1 × PC3 and PC2 × PC3) of the wine samples generated from a covariance matrix.

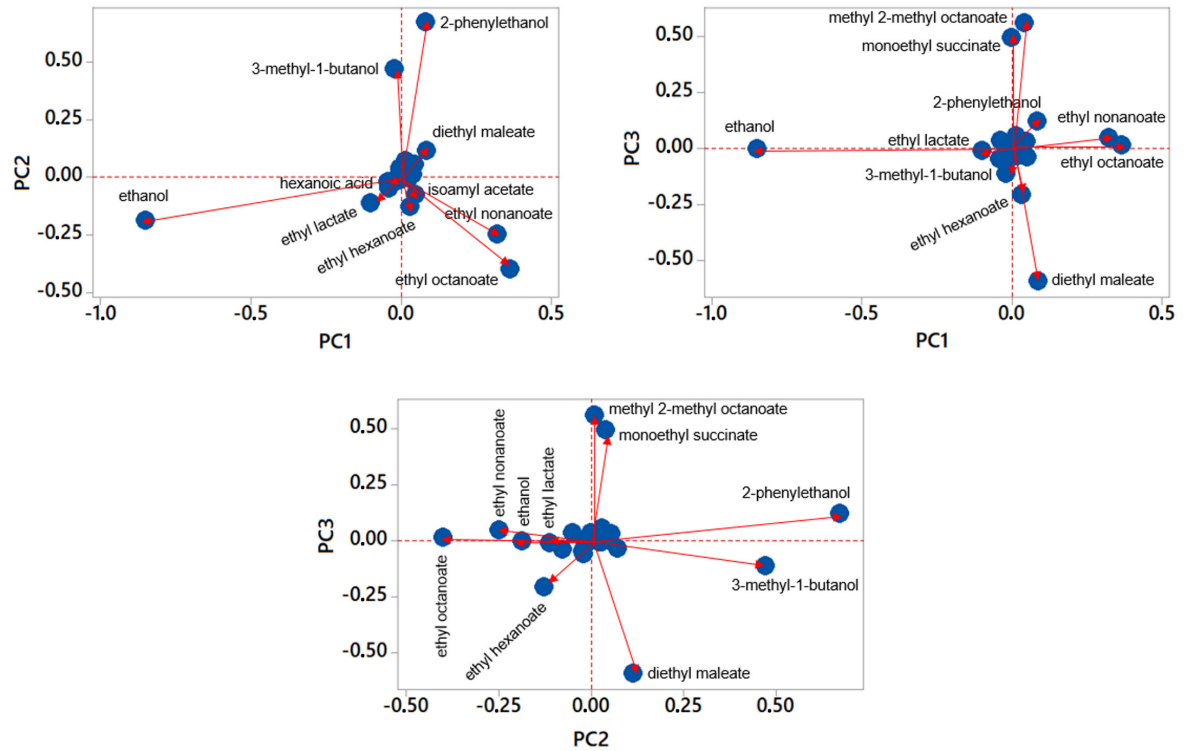

Figure 2. PCA loading plots (PC1 × PC2, PC1 × PC3 and PC2 × PC3) of the volatile compounds from the wine samples.

contents, moderate to high contents of ethyl hexanoate and diethyl maleate, and low contents or the absence of monoethyl succinate and methyl 2-methyl octanoate. PC2 × PC3, in turn, presented samples of the Cabernet Sauvignon, Cabernet Franc, Pinot Noir and Tannat wines distributed throughout the PC2. In general, these samples were poor in monoethyl succinate and methyl 2-methyl octanoate and presented varying levels of diethyl maleate and ethyl octanoate.

Finally, the present data allowed for the verification of a differentiated profile of the Merlot wines by the use of the Principal Components Analysis, largely due to the high contents of methyl 2- monoethyl succinate and methyl octanoate and the low levels of ethyl hexanoate and diethyl maleate. This differentiation was complemented by the presence of more specific volatile compounds and by the greater number of averages of significantly different relative areas.

4 Conclusions

In this study, it was also verified that SPME and GC-MS were shown to be precise and accurate techniques for the rapid identification of volatile compounds in the wines. These techniques can be successfully used in the determination of the flavour components and can be considered as simple, efficient and environment-friendly methods. The present study confirms a previous report that the volatile profile of red wines is mainly characterized by esters and higher alcohols. Wine aroma is one of the most important parameters responsible for its quality, and hence for consumer acceptance, but little is known about the volatile profile of Brazilian wines. Considering the continuous growth of Brazilian wine production and the importance of volatile compounds to their flavour, characterization studies are essential to improve the method of production of these beverages. Thus the present results open perspectives for future research on possible indicators that may serve for certification, as well as for the quality control of Brazilian red wines. Therefore additional studies using samples produced in different regions and with distinct production techniques are necessary to better characterize volatile organic compounds in Brazilian wines.

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