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Aromatic profile of Canaiolo nero wines in Santa Catarina highlands, Brazil

Perfil aromático de vinhos 'Canaiolo Nero' em regiões de altitude de Santa Catarina

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ABSTRACT

Highlands are a new frontier to produce fine wines in the Southern region of Brazil (27º 12' 24" S, 51º 06' 96" W, 1211 m altitude) and has presented specific oenological characteristics with a good adaptation of some varieties, among them, Canaiolo nero. To characterize the wines of this region, during the 2012 and 2014 harvests the volatile compounds were determined through the MHS-SPME-GC-MS technique. The grapes were harvested, and samples analyzed in triplicate. The positive identification of the volatile compounds was performed by comparing the experimentally obtained mass spectrum and retention index with the reference spectra and retention indices available in the literature. Four consecutive extractions were used for quantitative evaluation to avoid a matrix effect. Odor activity value was calculated from the perception threshold for each evaluated compound. The mean soluble solids content was 21.55º Brix, and total acid content was 81.0 meq L^{-1}; the mean weight of the clusters was 176 g, and the productivity was 2.2 tons per hectare (espalier – 1.5 m × 3.0 m). Thirty-one volatile compounds were identified and quantified in the analyzed samples. The major components were the aromatic alcohol 2-phenylethanol (38.364 µg L^{-1}), and the esters diethyl succinate (6.357 µg L^{-1}) and ethyl acetate (2.005 µg L^{-1}); and the compound of class C6, 1-hexanol (3.2 µg L^{-1}). Odor activity values showed the compounds that contribute the most to the aroma of the analyzed wines, highlighting ethyl isovalerate (OAV 394.38), ethyl hexanoate (OAV 9.22), ethyl cinnamate (OAV 8.62) ethyl isobutanoate (OAV 5.59), β-damascenone (OAV 2.44), hexanoic acid (OAV 4.03), octanoic acid (OAV 3.64) and isoamyl acetate (OAV 3.01). These results showed the aroma characteristics of Santa Catarina wines of the Canaiolo nero variety, especially fruity aromas of apple, green apple, strawberry, plum and banana; and floral aroma of violet and roses.

KEYWORDS: *Vitis vinifera,* odor activity value, chromatograph, threshold, volatile compounds.

RESUMO

As regiões de altitude elevada são uma nova fronteira para a produção de vinhos finos no Sul do Brasil (27º12'24" S, 51º06'96" W, 1211 m de altitude) por apresentar características enológicas diferenciadas com uma boa adaptação de algumas variedades, entre elas Canaiolo Nero. Para caracterizar os vinhos desta região, durante as colheitas 2012 e 2014 foram determinados os compostos voláteis por meio da técnica de MHS-SPME-GC-MS. As uvas foram colhidas, as amostras analisadas em triplicada e a identificação positiva dos voláteis foi realizada comparando o espectro de massa e o índice de retenção experimentalmente obtidos com os espectros de referência e os índices de retenção disponíveis na literatura. Para a avaliação quantitativa foram utilizadas quatro extrações consecutivas, para evitar o efeito da matriz e o valor da atividade do odor foi calculado a partir do limiar de percepção para cada composto avaliado. Os sólidos solúveis médios foram 21,55° Brix e acidez total de 81,0 meq L^{-1}; o peso médio dos cachos foi de 176 g e a produtividade de 2,2 toneladas por hectare (espaldeira - 1,5 m x 3,0 m). Um total de 31 voláteis foram identificados e quantificados nas amostras analisadas. Os principais componentes foram os ésteres álcool 2-feniletanol (38,364 µg L^{-1}), succinato de dietilo (6,357 µg L^{-1}) e acetato de etilo (2,005 µg L^{-1}); e o composto da classe C6, 1-hexanol (3,2 µg L^{-1}). Os valores da atividade do odor mostraram os compostos de maior contribuição para o aroma dos vinhos analisados, destacando o isovalerato de etila (OAV 394,38), o hexanoato de etila (OAV 9,22), o isobutanoato de etila e cinamato de etila (OAV 8,62) (OAV 5,59), β-damascenona (OAV 2,44), ácido hexanóico (OAV 4,03), ácido octanóico (OAV 3,64) e acetato de isoamila (OAV 3,01). Estes resultados mostraram o caráter aromático de vinhos da variedade Canaiolo Nero produzidos em Santa Catarina, especialmente aromas frutados de maçã, maçã verde, morango, ameixa e banana; e aroma floral de violeta e rosas.

PALAVRAS-CHAVE: *Vitis vinífera,* valor de odor ativo, cromatografia, limite, compostos voláteis.

INTRODUCTION

Canaiolo is a Tuscan black-skinned grape variety that was the main constituent of Chianti wine until the late 19th century. This grape variety is still grown nowadays throughout Central Italy, being used as a blending grape in a wide range of Sangiovese-based wines. Only a few Canaiolo wines are still produced; however, interest in this largely forgotten variety is growing. This variety was once extensively planted in Tuscany, Lazio, Marche, and Sardinia, and it still exists, although to a limited extent, in these regions (BANDINELLI et al. 2012). Canaiolo grape culture was tested in Brazil and showed good environmental adaptability, producing red wines tending to orange, and aromatic characteristics that aroused interest. In this sense, this variety was indicated for planting in Santa Catarina highlands, becoming an additional option for vine growers, whom can vinify it alone or in blends, mainly with the Sangiovese variety.

The volatile fraction significantly influences the aroma of wine and is considered one of the most important characteristics of the product quality and consumer acceptance. Over 1000 volatile compounds have been identified in wine, covering a wide range of polarity, solubility, and volatility characteristics. These compounds are present in variable concentrations, ranging from $ng.L^{-1}$ to $g.L^{-1}$ (BARROS et al. 2012).

The olfactory impact of each volatile compound depends on its concentration and its threshold of perception by the human nose, which varies considerably for each compound. Thus, the calculation of the odor activity value (OAV) is important for studying chemical compounds capable of stimulating a sensory response by the human olfactory system (JIANG et al. 2013).

In red wines, the volatile compounds are present in a complex matrix and can be associated with other wine compounds, such as polyphenols, polysaccharides, and ethanol, which may modify their volatility (POZO-BAYÓN & REINECCIUS 2009). In wine analysis, the headspace solid-phase microextraction (HS-SPME) technique has been widely used for the determination of volatile compounds. However, due to the high level of ethanol and other main volatile compounds, the recovery of analytes can be very low, in addition to being strongly dependent on the matrix (NOGUEROL-PATO et al. 2009, ANDUJAR-ORTIZ et al. 2009, ROBINSON et al. 2009), so that precision can only be guaranteed by using deuterated internal standards (ZAPATA et al. 2012). To overcome the matrix effect, multiple extractions in the HS-SPME technique (MHS-SPME) is a possible approach for the quantification of volatile compounds in complex matrices. MHS-SPME is an effective extraction procedure in which the same sample is subjected to a consecutive series of extractions at equal time intervals. Thus, the total peak area of the analytes can be determined by a single extraction, based on the geometric progression obtained from the consecutive extractions previously applied (COSTA et al. 2013).

According to PORRO & STEFANINI (2016), the highlands are a new frontier to produce fine wines in Southern Brazil and has presented specific oenological characteristics with a good adaptation of some varieties, among them, Canaiolo nero. According to data available in the literature, this is the first study to apply a quantitative method using MHS-SPME with gas-chromatography mass spectrometry (GC-MS) to research the volatile fraction of Canaiolo nero wines produced in Southern Brazil. Then, this study aimed to quantify volatile compounds that are important in Canaiolo wines produced in Santa Catarina highlands, during the 2012 and 2014 harvests.

MATERIAL AND METHODS

Samples

Samples of red wines of the Canaiolo nero variety, produced by micro vinification, were used in this study. Wines from the 2012 and 2014 harvests from a winery located in the municipality of Marari, Tangará in Santa Catarina, Brazil (1059 meters altitude, latitude 27º 12' 24" S and longitude 51º 06' 96" W).

Grapes of the Canaiolo variety were harvested when they reached physiological maturation and were transported to the Experimental Station of EPAGRI Videira, Santa Catarina. The grapes were separated from the bunches and kept in a stainless steel tank (20 L). The maceration period encompassed ten days, with two daily reruns. The liquid was separated from the solid parts and transferred to a stainless steel tank.

Before starting the alcoholic fermentation, sulfite addition (10 mg L^{-1} free SO_2) was conducted, and *Saccharomyces cerevisiae* strains (20 g / 100 kg) were added. The decarboxylation of malic acid by lactic acid bacteria occurred spontaneously, without inoculum addition. After alcoholic fermentation, the wines were stabilized in a cold room for 20 days and were added with free SO_2 (40 mg L^{-1}), and then bottled. Three bottles of each replicate were used for analysis.

Reagents and solutions

The analytical standards of the volatile compounds studied were obtained from Sigma-Aldrich (St. Louis, MO, USA), with purity equal to or greater than 98%. Ethanol and sodium chloride were purchased from LabSynth (Diadema, SP, Brazil). Ultrapure water was obtained through a Milli-Q purification system (Millipore, Bedford, MS, USA). For each studied compound, a 100 mg L^{-1} stock solution was prepared in 50% ethanol and stored at 4 °C. Standard solutions were prepared in synthetic wine (5 g L^{-1} tartaric acid, 11% ethanol, 3.5 pH). Total acidity (meq L^{-1}) and soluble solids (°Brix) were determined according to the International Organisation of Vine and Wine methods (OIV 2011).

MHS-SPME

The conditions associated with the MHS-SPME technique applied for the analysis of volatile compounds in Canaiolo nero wine samples were based on previous HS-SPME methodology optimization experiments. The experiments were conducted using 50/30 µm DVB/CAR/PDMS fiber, obtained from Supelco (Bellefonte, PA, USA). In each assay, 1.5 g NaCl and 5.0 mL sample were added in a 20 mL vial. The samples were incubated for 5 minutes at 56 °C, following, the fiber was exposed in the headspace for 55 minutes. Four sequential extractions were performed. After each extraction, the compounds were desorbed on the gas chromatographic injector for 6 minutes at 265 °C in splitless mode. The interval between extractions was 40 minutes.

Chromatographic conditions

Chromatographic analyses were performed on a Varian CP-3800 GC-IT/MS (USA) equipped with an ion trap analyzer (Varian Saturn 4000, USA), using the software MS Workstation software (version 5.4). The ion trap detector functioned at 200 °C in the transfer line, 50 °C in the manifold and 180 °C in the trap. All mass spectra were obtained by electron impact (EI), in the scan mode (25 – 400 m/z). The emission current was 50 µA, with 25000 s maximum ionization. The positive identification of compounds was performed by comparing the retention time obtained for the sample with the time observed for the standards of volatile compounds injected under the same conditions, and also based on the comparison of the obtained mass spectra with those found in the spectral database of the National Institute of Standards and Technology (NIST) MS 05, considering similarity above 70%. The retention index (linear temperature programmed retention indices – LTPRI) was calculated using a commercial hydrocarbon mixture (C_8 - C_{20}).

Chromatographic separation was performed using a ZB-WAXplus column (60 m x 0.25 mm x 0.25 µm) (Zebron, USA) and nitrogen gas as the carrier gas at 1.0 ml.min^{-1} flow rate. The temperature program was set according to REVI et al. (2014) with modifications: 40 °C initial oven temperature for 5 min, increasing by 2 °C per min until reaching 220 °C.

The odor activity value (OAV) was calculated as the ratio between the concentration of each compound and the threshold reported in the literature.

Statistical analysis

Analysis of variance (ANOVA) was applied to the experimental data, considering a 5% significance level ($p < 0.05$). Statistical analysis was made using the Statistica software, version 10 (STATSOFT Inc., Tulsa, OK, USA).

RESULTS AND DISCUSSION

The state of Santa Catarina, Brazil, is an emerging wine-producing area and several research have been conducted for varieties adapted to edaphoclimatic conditions of these regions to produce fine wines. Given this context, the assessment of the Canaiolo nero variety showed productivity between 2.09 and 2.63 tons per hectare, soluble solids (ºBrix) between 22.48 and 18.70, and total acidity (meq L^{-1}) between 71.4 and 90.6 (Table 1). These values are considered interesting for local conditions, showing high concentration of soluble solids in the 2012 harvest, but reduced values in 2014 due to excessive rainfall. Total acidity in both harvests showed satisfactory and adequate results for the elaboration of quality fine quiet wines.

Considering the importance of flavor compounds in wine quality, 31 volatile compounds in total were identified and quantified in Canaiolo nero from the Marari region, Southern Brazil. Seventeen esters were quantified in the analyzed samples, among which, most were ethyl lactate, diethyl succinate, ethyl acetate and ethyl octanoate (Table 2). Ethyl lactate and diethyl succinate are present in high concentrations in wines that undergo the malolactic fermentation process, since the esterification of lactic acid and succinic acid with ethanol occurs during the decarboxylation of malic acid by lactic acid bacteria (PERESTRELO et al. 2006, LIU et al. 2015). Ethyl acetate and ethyl succinate were present in higher concentrations in wines from the 2012 harvest, while ethyl lactate and ethyl octanoate were found in the 2014 harvest.

Table 1. Physical-chemical and productive characteristics of Canaiolo grapes in the 2012 and 2014 harvests.

Year	Sample	Soluble solids (°Brix)	Total acidity (meq L^{-1})	Yield ($t\ ha^{-1}$)
2012	1	22.24	81.3	1.32
	2	22.34	69.2	2.13
	3	23.24	68.1	3.69
	4	21.24	67.1	2.10
	5	23.34	71.2	1.19
	μ	22.48	71.4	2.09
	ơ	0.856	5.761	0.995
2014	1	18.72	92.8	2.63
	2	18.72	99.8	5.38
	3	17.82	86.7	2.33
	4	18.52	83.0	1.88
	5	19.72	90.6	0.94
	μ	18.70	90.6	2.63
	ơ	0.680	6.367	1.662

Ethyl isovalerate, ethyl cinnamate, ethyl hexanoate, ethyl isobutanoate, ethyl laurate and ethyl 2-methylbutanoate are among the ethyl esters that can be identified by the human nose at mean concentrations, as can be seen in Table 2. Ethyl isovalerate, ethyl isobutanoate, and ethyl 2-methylbutanoate, with fruity aromas of apple, banana, and strawberry, respectively, are branched-chain ethyl esters. Ethyl cinnamate, with honey, cinnamon, and floral odor, was present in greater concentration in wines from the 2014 harvest. Ethyl laurate and ethyl hexanoate were the ethyl esters of fatty acids found in higher concentration in this study. They are responsible for the waxy and green apple aromas, respectively. According to ANTALICK et al. (2014), the composition of fatty acids and esters of fatty acids in fermented beverages depends on the yeast strain used during fermentation.

Five higher alcohol acetates were quantified in the analyzed samples, but only isoamyl acetate presented mean concentrations greater than the olfactory perception threshold (Table 2). This compound has a banana aroma. According to GAMBETTA et al. (2014), the concentration of higher alcohol acetates is affected by vinification conditions. The yeast strain used in the fermentation and nutrient content of must are possibly the most important factors for the formation of acetates, since their synthesis depends on the availability of unsaturated fatty acids and the ratio of carbon and nitrogen available in the fermentation medium.

Major alcohols in Canaiolo nero varietal wines were 2-phenylethanol, 3-methyl-1-butanol, and 1-propanol (Table 2). The content of 1-propanol and 3-methyl-1-butanol in wine is critically influenced by the yeast strain responsible for the beginning of alcoholic fermentation. On the other hand, 2-phenylethanol is probably formed from phenylalanine through the shikimic acid route (ROBINSON et al. 2014), and its concentration in wine depends on the precursor amino acid. Considering the analyzed alcohols, only 2-phenylethanol had a mean concentration above the olfactory perception threshold. This alcohol contributes to the aromas of roses and honey.

Considering the family of fatty acids, seven compounds were detected and quantified in the studied wines, including small (propanoic), medium (hexanoic, octanoic and decanoic and 10-undecenoic) and branched-chain (isobutyric and isovaleric) fatty acids. These compounds may originate in grapes or may be formed during fermentation by microorganisms (PEREIRA et al. 2014). Among them, the major components were octanoic, isovaleric and isobutyric acids (Table 2). These compounds may be formed via yeast anabolic route or β-oxidation of long-chain fatty acids. In addition, fatty acids are involved in the esterification reactions that lead to the formation of esters, and their decrease during maceration and alcoholic fermentation of wines is typical (PETROPULOS et al. 2014). Regarding the impact of fatty acids on the aroma of the studied wines, it was found that hexanoic, octanoic and decanoic acids presented mean OAV >

1. These compounds contribute to sweet, cheese and rancid odors.

Table 2. Concentration of volatile compounds (µg L^{-1}), threshold, OAV and descriptive odor of samples of Canaiolo nero wines produced in Marari, Southern Brazil.

Compound	Concentration (µg L^{-1})		OT	OAV		Odor descriptor
	2012	2014		2012	2014	
Ethyl acetate	2817.369	1193.861	12000	0.2348	0.0995	Solvent fruity balsamic
Ethyl isobutanoate	85.461	40.453	15	5.6974	2.6969	Fruity, banana
Isobutyl acetate	69.691	59.888	1600	0.0436	0.0374	Fruity, apple, banana
Ethyl butanoate	69.691	62.333	400	0.1742	0.1558	Fruity Strawberry
Ethyl 2-methylbutanoate	38.778	162.014	18	2.1543	9.0008	Strawberry, candy fruit
Ethyl isovalerate	191.290	507.307	1	191.2903	507.3070	Fruity, Apple
Isoamyl acetate	70.764	69.565	30	2.3588	2.3188	Banana
1-propanol	388.775	2473.854	306000	0.0013	0.0081	Ripe fruit, alcohol
Ethyl hexanoate	53.376	149.635	14	3.8126	10.6882	Fruity, green Apple
Hexyl acetate	24.906	29.809	670	0.0372	0.0445	Herbs, apple, pear
3-methyl 1-butanol	1371.524	5587.137	30000	0.0457	0.1862	Burnt, alcohol, nail
1-hexanol	40.800	19.631	110	0.3709	0.1785	Herbaceous, resinous
Ethyl octanoate	110.228	166.592	580	0.1900	0.2872	Fruity, abacaxi
Ethyl nonanoate	21.540	18.522	1300	0.0166	0.0142	Fruity, Floral
Isovaleric acid	359.075	2314.994	3000	0.1197	0.7717	Cheese, rancidity
Ethyl decanoate	11.645	100.587	200	0.0582	0.5029	Fruity, grape
Propanoic acid	41.058	109.541	8100	0.0051	0.0135	Cheese
β-damascenone	0.642	23.828	0.05	12.8351	476.5598	Baked apple, floral, honey
α-terpineol	38.109	70.609	250	0.1524	0.2824	Floral, candy, anise, mint
Diethyl succinate	7919.409	4796.136	200000	0.0396	0.0240	Wine, caramel, fruity
Phenylethyl acetate	90.502	22.022	250	0.3620	0.0881	Roses, floral, honey
Ethyl Laurate	9.500	9.787	1.5	6.3333	6.5250	Waxy
Caproic acid	153.618	209.540	8	19.2023	26.1924	Waxy
2-phenylethanol	32301.907	44487.297	14000	2.3073	3.1777	Roses, honey
Isobutyric acid	780.804	4649.546	2000000	0.0004	0.0023	Fatty
Octanoic acid	7013.793	14299.467	500	14.0276	28.5989	Rancidity, candy, cheese
γ-nonalactone	58.067	949.849	30	1.9356	31.6616	Coconut, peach
Ethyl lactate	11032.447	37039.682	150000	0.0735	0.2469	Acid, medicinal, strawberry
Ethyl cinnamate	4.194	11.999	1.1	3.8125	10.9079	Honey, cinnamon, floral
Capric acid	163.927	281.935	6	27.3212	46.9892	Waxy
10-undecenoic acid	644.345	254.050	-	-	-	-

Only one terpene was quantified in the studied wines, α-terpineol (Table 2). Having a characteristic aroma of anise and mint, α-terpineol was present in lower concentrations in the 2012 samples, and this behavior may be related to the occurrence of acid-catalyzed reactions during wine aging, which transform α-terpineol into 1,8-terpenes (PEREIRA et al. 2014).

Lactones are formed during the alcoholic fermentation through the action of the yeasts or accumulated during the aging of the wine (PEREIRA et al. 2014). In this study, only γ-nonalactone was evaluated in the Canaiolo nero wine samples, and its concentration was significantly different ($p<0.05$) considering the harvest, with higher levels in 2014. This compound has important sensory properties, contributing to coconut and peach aromas.

Β-Damascenone is a compound formed from the oxidative cleavage of carotenoids, which occurs during grape maturation (GAMBETTA et al. 2014); thus, being responsible for the varietal aroma. ANOVA showed that β-damascenone content presents differences of concentration according to the harvest, being higher in 2014. When considering the mean level of β-damascenone in Canaiolo nero wine samples, we can note its contribution to the aroma, with high values of OAV. This compound is a potent odorant and can be characterized by odors of cooked apple, flowers, and honey.

Based on OAV data obtained from the analyzed wines, it can be seen that the Canaiolo nero wines produced in the Marari region, Southern Brazil, present sweet and floral (β-damascenone and 2-phenylethanol), apple (ethyl isovalerate), green apple (ethyl hexanoate), banana (isoamyl acetate and ethyl isobutanoate), strawberry (ethyl 2-methylbutanoate) and coconut (γ-nonalactone) odors, in addition to wax and cheese odors (medium-chain fatty acids).

CONCLUSION

The MHS-SPME-GC-MS technique allowed the quantification of a group of volatile compounds that contribute substantially to the aroma of red wines, showing the importance of esters, alcohols and fatty acids for the aroma of the Canaiolo nero wines produced in the region of Marari, Southern Brazil. Considering the OAVs, the descriptors fruity, sweet, waxy and floral are the most relevant to characterize the aroma of the studied wines. Differences in the composition were evident among the samples, demonstrating the effects of climatic conditions of each harvest on the metabolism of volatile compounds. This is the first characterization study of Canaiolo nero wines produced in high-altitude regions of Southern Brazil. Further studies are needed to better understand the chemical composition and sensorial profile, and to better characterize the wines produced in this new frontier of wine production in Brazil.

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