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Analytical and Sensorial Characterization of the Aroma of Wines Produced with Sour Rotten Grapes Using GC-O and GC-MS: Identification of Key Aroma Compounds

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ABSTRACT: In the present work, the aroma profiles of wines elaborated from sound and sour rot-infected grapes as raw material have been studied by sensory analysis, gas chromatography−olfactometry (GC-O), and gas chromatography−mass spectrometry (GC-MS), with the aim of determining the odor volatiles most likely associated with this disease. The effect of sour rot was tested in monovarietal wines produced with the Portuguese red grape variety Trincadeira and in blends of Cabernet Sauvignon and sour rotten Trincadeira grapes. Wines produced from damaged berries exhibited clear honey-like notes not evoked by healthy samples. Ethyl phenylacetate (EPhA) and phenylacetic acid (PAA), both exhibiting sweet honey-like aromas, emerged as key aroma compounds of sour rotten wines. Their levels were 1 order of magnitude above those found in controls and reached 304 and 1668 μg L^{-1} of EPhA and PAA, respectively, well above the corresponding odor thresholds. Levels of γ-nonalactone also increased by a factor 3 in sour rot samples. Results also suggest that sour rot exerts a great effect on the secondary metabolism of yeast, decreasing the levels of volatiles related to fatty acids and amino acid synthesis. The highest levels of γ-decalactone of up to 405 μg L^{-1} were also found in all of the samples, suggesting that this could be a relevant aroma compound in Trincadeira wine aroma.

KEYWORDS: sour rot, wine, aroma, flavor, Trincadeira, GC-O, GC-GC, ethyl phenylacetate, phenylacetic acid, γ-lactones

INTRODUCTION

Fruit rots often determine harvest date and influence grape quality in warm and humid grape-growing regions.[1] The cause of grape sour rot, appearing 3−4 weeks before ripening, is the combined activity of three groups of factors: (a) primary factors (insects, birds, diseases such as mildews, grape berry moth attacks (*Lobesia botrana*), mechanical and physiological injuries), which damage the berry skin; (b) secondary microorganisms (bacteria, yeasts, and other fungi) that penetrate the broken skins of the injured berries; and (c) secondary insects (*Drosophila* flies and beetles) quickly attracted by the rotting and fermenting grapes, enhancing the process and accelerating its spread throughout the entire cluster.[2,3] Sour rot is characterized by the main role of yeasts in the rotting process. The most frequently isolated yeast species from sour rot-damaged grapes are *Hanseniaspora uvarum*, *Candida stellata*, *Metshnikowia pulcherrima*, *Candida krusei*, and *Kloeckera apiculata*,[4,5] and *Zygoascus hellenicus* and *Issatchenkia occidentalis*.[6] Other microorganisms, especially the bacteria *Acetobacter*, are involved in sour rot infection. Among the transformations carried out by acetic acid bacteria, the most important is the oxidation of ethanol into acetic acid. Indeed, sour rot owes its name to the strong and pungent odor of acetic acid (vinegar) present on rotten grapes and is characterized by grape pulp browning, disaggregation of the internal tissues, detachment of the rotten berry from the pedicel, and grape dropping.[5,7] In hot climates, *Botrytis cinerea* has occasionally been isolated from grape sour rot.[8] However, sour rot development is not dependent on *B. cinerea* infection,[9] in contrast to the predominant role of this fungal pathogen on bunch rot in regions with relatively cool fruit-ripening conditions.

Sour rot affects both crop yield and wine quality. It is commonly accepted between winemakers that the vinification of damaged grapes is associated with the production of low-quality wines with weak storage/aging potential.

The diverse group of organisms involved in sour rot is known to alter fruit composition as a result of the production of high levels of a wide range of metabolites including acetic acid, glycerol, ethyl acetate, ethanol, acetaldehyde, and galacturonic and gluconic acids.[10−12] In the late 1970s, Loinger et al.[13] tried to clarify for the first time the consequences of sour rot on wine quality, investigating the effect of the disease on the sensorial characteristics of wines from the Semillon grape variety. This study showed that clusters with 20−40% rotten berries resulted in a clear and marked reduction in wine quality, whereas wines from clusters with 80% rot were totally rejected. Later, Zoecklein et al.[10] determined the influence of sour rot on white Riesling must monoterpenic composition. Sour rot had no influence on free or bound volatile terpenes, but did alter the terpene profile by reducing monoterpene alcohols, whereas monoterpene oxides increased. Trincadeira is a neutral *Vitis vinifera* L. red native grape variety widely planted in Portugal,[14] highly susceptible to

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Table 1. Wines Analyzed in the Experiment Including Grape Varieties, Health Status, Wine Production Phase, and Some Basic Compositional Parameters Determined by FTIR and Analyses Performed for Each Wine Sample

		grape[a] (% w/w)										
trial	wine sample	S (T)	S (CS)	SR (T)	wine phase	alcohol (% v/v)	pH	volatile acidity[b] (g L^{-1})	residual sugar (g L^{-1})	TPI[c]	CI[d]	analyses
T0[e]	T0 MLF	100			MLF[f]	12.3	3.63	0.10	1.6	33.5	17.0	GC-O; chemical;[g] sensory
	T0 BOT	100			BW[h]	11.4	3.62	0.12	1.4	30.8	14.1	GC-O; chemical; sensory
T30	T30 MLF	70		30	MLF	14.1	3.54	0.36	3.2	35.9	15.9	sensory
	T30 BOT	70		30	BW	13.3	3.52	0.30	2.6	29.6	12.6	sensory
T50	T50 MLF	50		50	MLF	14.6	3.54	0.45	4.1	39.4	20.1	GC-O; chemical
	T50 BOT	50		50	BW	13.9	3.54	0.46	3.8	39.0	14.8	GC-O; chemical
CT0[e]	CT0 MLF	30	70		MLF	11.9	3.57	0.11	0.7	36.5	16.8	GC-O; chemical; sensory
	CT0 BOT	30	70		BW	12.1	3.57	0.15	0.6	37.8	18.3	GC-O; chemical; sensory
CT30	CT30 MLF		70	30	MLF	13.3	3.53	0.33	2.0	39.7	17.5	GC-O; chemical; sensory
	CT30 BOT		70	30	BW	12.8	3.55	0.33	1.9	39.4	14.8	GC-O; chemical; sensory

[a] S(T), sound Trincadeira; S(CS), sound Cabernet Sauvignon; SR(T), sour rot Trincadeira. [b] Expressed as acetic acid. [c] TPI, total phenols index, expressed in absorbance ($A_{280\ nm}$) × 100. [d] CI, color intensity, expressed as ($A_{420\ nm} + A_{520\ nm} + A_{620\ nm}$) × 5. [e] Control set vinifications. [f] MLF, after malolactic fermentation. [g] Major (liquid−liquid microextraction and GC-FID) and minor (SPE and GC-ion trap-MS) compounds. [h] BW, bottled wine (after 16 months of bottle aging).

cryptogamic diseases, and particularly sensitive to sour rot.[15] Concerning the aroma characterization of Trincadeira wines, Cabrita et al.[16] studied the composition of the glycosidic flavor precursors extracted from Trincadeira grape samples, as well as the fermentative compounds and glycoconjugated aroma compounds (monoterpenes and norisoprenoids) present in the respective wines.[17] Goreti et al.[14] investigated the differences in odor-active compounds of Trincadeira wines obtained from five different certified clones, using gas chromatography−olfatometry (GC-O).

Previous studies performed by Escudero et al.[18] and more recently by San-Juan et al.[19] have evidenced that, by using a dynamic headspace technique, based on a purge-and-trap headspace solid-phase extraction (HS-SPE) system in the preparation of wine extracts for GC-O, it is possible to obtain relatively simple and clean olfactograms and to establish a hierarchy of the most important odorants according to their potential sensory impact. The GC-O technique strongly contributed in the past decade to the overall identification of odor-active compounds in white and red musts and/or young and aged wines. In fact, GC-O analysis has been widely used to identify odor-active compounds in wines made from Chardonnay,[20,21] Riesling,[22] Gewurztraminer,[23−25] Merlot and Cabernet Sauvignon,[26−28] Grenache and Tempranillo,[26,29−32] Zalema,[33] Palomino Fino,[34] Touriga Nacional,[35] Aragonez,[36,37] and Trincadeira [14] grape cultivars. This technique has also been applied to screen the odor volatile composition of sweet wines obtained from overripe berries affected by *B. cinerea*. These works have been especially devoted to noble rotten wines from the Sauternes (France) region,[38−40] although some other examples such as Fiano wines from the Amarone (Italy) region can be found in the literature.[41] Despite the existing investigations on botrytized wines (noble rot), to our knowledge, no work focused on the aroma profile of wines affected by sour rot has been published to date, which means that the number and nature of the odorants associated with this grape disease are not known.

Therefore, the main goal of this research was to compare the volatile composition and sensory profiles of wines produced with sound and sour rot affected grapes. For this purpose, the methodological approach combines GC-O and chemical quantitative analysis of major and minor compounds, together with sensory descriptive analysis, to understand the role of sour rot in the odor nuances of these wines.

MATERIALS AND METHODS

Wines. During the 2008 vintage, healthy (sound) and sour rot affected bunches (Trincadeira and Cabernet Sauvignon grape varieties) were collected at the time of harvest from an experimental vineyard at the Instituto Superior de Agronomia (Lisbon, Portugal, latitude 38° 42′ 31.57″ N and longitude 9° 11′ 14.01″ W) and transported to the experimental cellar in 20 kg plastic boxes. Infected bunches were collected at the same stage of infection. Five microvinification sets (red style fermentation with grape skin contact) were carried out in 50 kg capacity stainless steel tanks using sound grapes to which different amounts of sour rot damaged grapes were added: two sets of monovarietal Trincadeira grape variety with 30 and 50% sour rotten grapes and one set of 70% sound Cabernet Sauvignon blended with 30% sour rotten Trincadeira grapes. Two additional control sets composed, respectively, by 100% sound Trincadeira grapes and 70% sound Cabernet Sauvignon blended with 30% sound Trincadeira grape cultivars were performed (Table 1). The grapes (40 kg per set) were mechanically destemmed and crushed on a commercial grape destemmer−crusher, followed by SO_2 addition (50 mg/kg) to emulate winery conditions and 18 h of pre-fermentative maceration at room temperature. Musts were inoculated with 10^6 cells/mL of selected commercial *Saccharomyces cerevisiae* yeast (Fermivin, DSM, Delft, The Netherlands). Fermentation temperature varied from 22 to 27 °C, and grape skins were submerged twice a day. Once malolactic fermentation (MLF) was complete, free SO_2 levels were corrected to 40 mg/L and wines were bottled without filtration in 0.75 L glass bottles capped with natural corks and kept at cellar temperature (18 ± 2 °C). For each produced

Table 2. Aroma Attributes Selected for Descriptive Sensory Analysis and Composition of the Corresponding Reference Standards

attribute	reference standard[a]
fresh fruit	3 mL each of pear, banana, strawberry, and blueberry juice
ripe fruit	1 mL of prune juice + 5 mL of brine from canned figs
vegetal/earthy	5 g of sliced bell pepper soaked for 15 min + 5 mL of brine from canned green beans
toasted	1 drop each of samples 48 (toast) and 49 (roasted almonds) of "Le Nez du Vin" Jean Lenoir
oxidized	5 mL of brine from canned potatoes
honey	half a teaspoon of honey
floral	1 drop of phenylethyl alcohol
chemical	2 mL of 95% ethanol + 1.5 mL of vinegar
spicy	pinch of black pepper + 2 cloves
butter	half a teaspoon of butter
animal	100 mg of shoe polish + 1 drop of sample 45 (leather) of "Le Nez du Vin" Jean Lenoir
reduction	1 drop each of samples 6 (sulfur) and 9 (cabbage) of "Le Nez du Vin" Jean Lenoir

[a] Quantities specified are those added to 40 mL of neutral red wine.

wine, two different phases within aging were analyzed: just after MLF and after 16 months of bottle aging in the cellar (BOT). Wine samples of each phase were stored at −20 °C until analysis. The detailed information of all wine samples analyzed in the experiment is shown in Table 1.

Reagents and Standards. The chemical standards were supplied by Aldrich (Gillingham, U.K.), Fluka (Buchs, Switzerland), Sigma (St. Louis, MO), Lancaster (Strasbourg, France), PolyScience (Niles, IL), Chemservice (West Chester, PA), Interchim (Monluçon, France), International Express Service (Allauch, France), and Firmenich (Geneva, Switzerland). LiChrolut EN resins (ethylvinylbenzene-divinylbenzene) and polypropylene cartridges were obtained from Merck (Darmstadt, Germany). Dichloromethane and methanol of LiChrosolv quality were from Merck; absolute ethanol and ammonium sulfate were from Panreac (Barcelona, Spain), and all of them were of ARG quality. Pure water was obtained from a Milli-Q purification system (Millipore, Bedford, MA). Semiautomated solid-phase extraction (SPE) was carried out with a VAC ELUT 20 station from Varian (Walnut Creek, CA).

Enological Parameters. All wines were analyzed by Fourier transform infrared spectroscopy (FTIR), in WineScan FT120 (Foss, Hillerød, Denmark) equipment. The following enological parameters were determined: ethanol content (% v/v), pH, volatile acidity, residual sugar, total phenol index (TPI), and color intensity (CI).

Sensory Descriptive Analysis. The sensory panel was composed of 7 females and 7 males, 24−70 years of age (mean = 36), all belonging to the laboratory staff and with long experience in sensory analysis. Four specific 1 h training sessions were carried out. In the first one, judges generated descriptive terms to define the wines of the study. In sessions two and three, different aroma standards were presented and discussed by the panel. From these discussions, 12 aroma attributes were retained as the most appropriate to describe the wine samples: fresh fruit, ripe fruit, vegetal/earthy, floral, spicy, honey, toasted/vanilla, butter, animal, chemical, reduction, and oxidized. The aroma reference standards used to define each of these attributes are presented in Table 2. In training session four, panelists scored the intensity of each attribute using a seven-point scale (0 = not detected, 1 = weak, hardly recognizable note, 2 = clear but no intense note, 3 = intense note); half values were allowed. After the training period, the eight wine samples were evaluated once during a formal session. Judges were asked to evaluate wines orthonasally and to rate the intensity of each attribute listed in Table 2 according to the 7-point scaled used during training. Sessions took place in individual booths and lasted approximately 45 min. A 10 min break was enforced in the middle of each session to limit judge fatigue. Samples were presented according to a William Latin-square arrangement. The data were processed to calculate the "modified frequency" parameter proposed by Dravnieks:[42] $MF\,(\%) = [F\,(\%) \times I\,(\%)]^{1/2}$, where $F\,(\%)$ is the detection frequency of an aromatic attribute expressed as percentage of total number of judges and $I\,(\%)$ is the average intensity expressed as percentage of the maximum intensity.

GC-O Study. Wine extracts were obtained by a dynamic headspace sampling technique. A standard SPE cartridge (0.8 cm internal diameter, 3 mL internal volume) filled with 400 mg of LiChrolut EN resins was first washed with 20 mL of dichloromethane and then dried by letting air pass through (negative pressure of 0.6 bar, 10 min). The cartridge was placed on the top of a flask containing 80 mL of wine kept at room temperature (approximately 21 °C). A stream of nitrogen (500 mL min^{-1}) was applied onto the surface of the liquid, purging the volatile compounds of the headspace. The volatile wine constituents released were trapped in the cartridge containing the sorbent. After 100 min, the cartridge was removed and dried by letting N_2 pass through; then, analytes were eluted with 3.2 mL of dichloromethane with 5% methanol. After this, the extract was concentrated under a stream of pure N_2 to a final volume of 200 μL.

GC-O analysis was carried out in a gas chromatograph Trace GC (Termoquest, Milan, Italy) with a flame ionization detector (FID) and an olfactometric port ODO-I from SGE (Ringwood, Australia). This instrument was equipped with a capillary column DB-WAX (polyethylene glycol) from J&W Scientific (Folsom, CA), 30 m × 0.32 mm i.d., 0.5 μm film thickness, and a precolumn (3 m × 0.32 mm i.d.) from Supelco (Bellefonte, PA). Chromatographic conditions were as follows: hydrogen as the carrier gas (3.5 mL min^{-1}); splitless injection (splitless time 60 s); injection volume, 1 μL; injector temperature, 250 °C; detector temperature, 250 °C. The oven temperature program was the following: 40 °C for 5 min, then raised at 4 °C min^{-1} to 100 °C and at 6 °C min^{-1} to 220 °C, followed by 20 min at 220 °C. The sniffing port was equipped with a humidified air makeup and sequentially heated using a laboratory-made rheostat to prevent condensation of high-boiling compounds.

A panel of six judges (two women and four men, ranging from 23 to 32 years of age), carried out the sniffing of the extracts. Prior to GC-O analysis, panelists followed a training period as described in ref 43. Sniffing time was approximately 40 min, and each judge carried out one session per day. The panelists were asked to provide a descriptor to characterize the eluted odor and to rate its intensity using a 7-point category scale (0 = no odor; 1 = weak odor, low intensity; 2 = clear perception of odor, strong intensity; 3 = extremely strong intensity of odor; intermediate values of 0.5, 1.5, and 2.5 were allowed). The quantitative capacity of this technique has been demonstrated elsewhere.[43] As a large number of odorants are at concentrations near the threshold in the headspace extracts, the data processed were a

mixture of the intensity and the frequency of detection of an odorant. This parameter is labeled "modified frequency" (MF) and is calculated with the formula proposed by Dravnieks:[42] MF (%) = $[F(\%) \times I(\%)]^{1/2}$, where $F(\%)$ is the detection frequency of an aromatic odorant expressed as percentage of total number of judges and $I(\%)$ is the average intensity expressed as percentage of the maximum intensity. The odorants were identified by comparison of their odors and chromatographic retention index in DB-WAX column with those of pure reference compounds.

Identification of Unknown Odorants. The isolation of seven unknown odorants detected in the GC-O experiment and for which the identity could not be achieved by the standard procedure was carried out by means of a multidimensional GC technique (GC-GC). Basically, in GC-GC a fraction of effluent from a primary column is isolated and reanalyzed by a second column of different stationary phase selectivity. We employed a dual GC-GC-MS system composed of two independent chromatographs interconnected by means of a Deans valve and a thermoregulated transfer line kept at 200 °C. The first chromatograph was equipped with DB-WAX column (polyethylene glycol), a FID, and an olfactometric port, and the second one was equipped with a FactorFour VF-5MS column (polymethylsiloxane−5% diphenyl) from Varian (Walnut Creek, CA), a mass spectrometry (MS) detector, and a second olfactometric port. The complete description of the system is given in ref 44. Concentrated wine extracts were injected on this dual system until a satisfactory mass spectrum for the target odorant could be obtained. The identity was finally confirmed by the injection of the pure reference standard in the GC-GC-MS system.

Chemical Quantitative Analysis. *Major Compounds (Liquid−Liquid Microextraction and GC-FID Analysis).* The quantification of major compounds was carried out using the method proposed and validated by Ortega et al.[45] The extract was prepared in accordance with this method with the following adjustments: in 15 mL screw-capped centrifuge tubes, containing 4.1 g of ammonium sulfate, were added 2.7 mL of wine, 6.3 mL of water, 20 μL of internal standard solution (2-butanol, 4-methyl-2-pentanol, 4-hydroxy-4-methyl-2-pentanone, heptanoic acid, and 2-octanol at 200 μg/mL in ethanol), and 0.25 mL of dichloromethane. The tubes were shaken for 90 min and then centrifuged at 2500 rpm for 10 min. Once the phases had been separated, the dichloromethane phase was recovered with a 0.5 mL syringe and transferred to a 0.3 mL vial. The extract was then analyzed by GC in a Hewlett-Packard 5890 series II gas chromatograph with FID. This instrument was equipped with a capillary column DB-WAX (30 m × 0.32 mm i.d. and 0.5 μm film thickness) from J&W Scientific preceded by a 2 m × 0.53 mm uncoated precolumn. Chromatographic conditions were as follows: hydrogen as the carrier gas (2.2 mL min^{-1}); split injection mode (1:10 split relation) with 3 μL injection volume; injector temperature at 250 °C, and detector temperature at 250 °C. The initial column temperature was 40 °C for 2 min, heated to 200 at 2 °C min^{-1}, and remaining at that temperature for 30 min. Quantitative data were obtained by interpolation of relative peak areas in the calibration graphs built by the analysis of synthetic wines containing known amounts of the analytes.

Minor Compounds (SPE and GC-Ion Trap-MS Analysis). This analysis was carried out using the method proposed and validated by López et al.[46] with the following changes in the previous procedure: standard SPE cartridges (1 mL total volume) filled with 50 mg of LiChrolut EN resins were placed in the vacuum manifold extraction system, and the sorbent was conditioned by rinsing the cartridges with 6 mL of dichloromethane, 2 mL of methanol, and, finally, 2 mL of a water−ethanol mixture (12%, v/v). The cartridges were then loaded with 15 mL of wine sample and 10 μL of a surrogate standards solution containing 3-octanone, β-damascone, and heptanoic acid (all at 200 μg/g of ethanol). This mixture was passed through the SPE cartridges (2 mL min^{-1}), followed by a wash step using 5 mL of 40% water−methanol solution. The resins were then dried by letting air pass through (negative pressure of 0.6 bar, 10 min). Analytes were recovered in a 2 mL vial, by elution with 0.6 mL of dichloromethane. Twelve microliters of an internal standard solution (300 mg L^{-1} of 4-hydroxy-4-methyl-2-pentanone and 2-octanol) was added to the eluted sample. The extract was then analyzed by GC with ion trap MS detection. A CP-3800 gas chromatograph fitted to a Saturn 2200 ion trap MS from Varian was used. Chromatographic analyses were performed under the conditions described in ref 46.

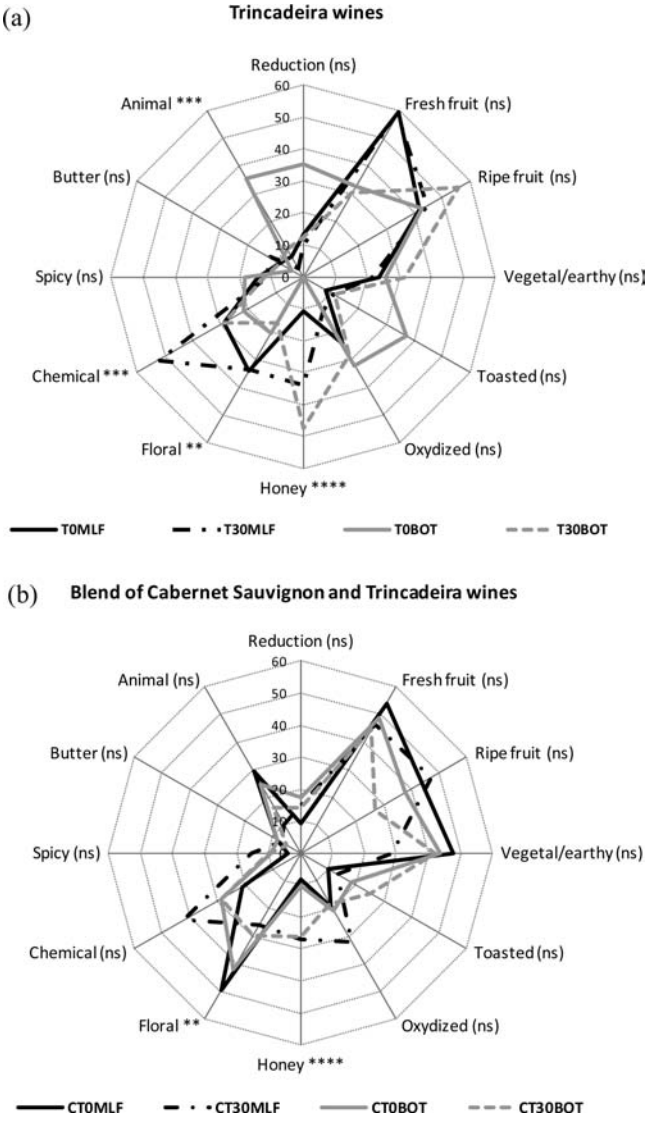

Figure 1. Graph of the mean sensory ratings MF (%) of the four Trincadeira (a) and Trincadeira/Cabernet Sauvignon (b) wines studied. Notations of ∗, ∗∗, ∗∗∗, and ∗∗∗∗ indicate significance at $p < 0.1$, $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively; ns indicates no significant difference.

Statistical Analysis. For the data obtained in the sensory descriptive analysis, a two-factor (wines and subjects) ANOVA was performed on the attributes' intensity scores derived from the four Trincadeira wines and the four blends of Trincadeira/Cabernet Sauvignon. To look for discriminant odorants, a two-factor (wine and subjects) analysis of variance (ANOVA) was performed on the individual intensity GC-O scores. For the chemical quantitative data, a two-factor ANOVA was carried out to check for significance of the factors "sour rot" and "time". These analyses were performed using SPSS software (version 15.0) from SPSS Inc. (Chicago, IL).

Table 3. Odorants Found by GC-O in the Eight Studied Wines[a]

LRI[b]	LRI[c]	odor description	identity	T0 MLF	T50 MLF	T0 BOT	T50 BOT	CT0 MLF	CT30 MLF	CT0 BOT	CT30 BOT	av (SD)	max−min
956		butter, cream	2,3-butanedione (diacetyl)[d]	91	83	88	83	83	73	78	75	82 (6.2)	18
1010	912	solvent, alcoholic	ni[e] 1010	0	0	10	41	10	31	29	26	18 (15.4)	41
1034		fruity, strawberry	ethyl butyrate[d]	67	50	53	55	60	65	60	58	59 (5.8)	17
1049		fruity, strawberry	ethyl 2-methylbutyrate[d]	65	55	75	71	71	63	76	73	69 (7.1)	21
1069		fruity, strawberry	ethyl 3-methylbutyrate[d]	68	61	73	71	76	59	75	71	69 (6.3)	17
1099		fusel	isobutanol[d]	19	29	41	20	26	45	41	41	33 (10.5)	26
1125		banana	isoamyl acetate[d]	54	33	35	55	65	68	43	33	48 (14.2)	35
1196		fruity, strawberry	ethyl 4-methylpentanoate[d]	29	19	30	0	48	22	29	19	25 (13.6)	48
1204		gas	(*E*)-2-hexenal[d]	0	0	22	10	0	0	33	7	9 (12.4)	33
1216	914	geranium, green	ni[e] 1216	58	24	27	47	0	0	0	0	20 (23.4)	58
1220		fusel	isoamyl alcohol[d]	90	85	88	85	90	90	93	87	89 (2.8)	8
1239		fruity, strawberry	ethyl hexanoate[d]	80	67	73	55	75	69	69	58	68 (8.4)	25
1302		mushroom	1-octen-3-one[d]	0	31	24	22	19	19	30	19	21 (9.6)	31
1334	982	coconut, sweet	2-heptanol[f]	0	0	24	17	26	12	36	0	14 (13.8)	36
1371		green, grass	1-hexanol[d]	0	14	0	30	0	0	0	0	6 (11.0)	30
1401	1010	rotten food, garlic, onion	dimethyl trisulfide[f]	0	0	0	25	0	0	0	0	3 (8.5)	24
1449		baked potatoes	methional[d]	33	0	31	22	31	19	12	14	20 (11.4)	33
1452		vinegar	acetic acid[d]	45	49	50	53	63	49	55	49	52 (5.5)	18
1503		aldehydic, rancidity	(*Z*)-2-nonenal[d]	0	0	31	12	0	33	12	19	13 (13.5)	33
1530		pepper, earthy	3-isobutyl-2-methoxypyrazine[d]	0	0	0	0	27	0	36	10	9 (14.4)	36
1632		cheese	butyric acid[d]	19	36	24	29	24	26	17	19	24 (6.2)	19
1675		cheese	isovaleric acid[d]	63	61	51	47	82	71	85	62	65 (13.5)	38
1718		baked vegetables	methionol[d]	0	0	0	0	33	29	17	10	11 (13.8)	33
1767	1041	geranium, green	ni[e] 1767	24	43	22	49	61	24	12	19	32 (17.1)	49
1782	1262	honey, sweet	ethyl phenylacetate[f]	0	0	0	47	0	7	0	10	8 (16.2)	47
1803		coconut, sweet, flowers	β-phenylethyl acetate[f]	43	45	43	49	10	22	12	22	31 (15.9)	39
1813		baked apple	β-damascenone[d]	41	43	29	24	33	31	41	17	32 (9.1)	26
1856		medicinal	2-methoxyphenol (guaiacol)[d]	29	19	26	24	24	29	12	36	25 (7.2)	24
1880		floral	ethyl dihydrocinnamate[d]	45	10	12	10	43	33	58	17	29 (18.8)	48
1909		rose	β-phenylethanol[d]	80	51	80	63	65	80	71	80	71 (10.8)	29
2040		burnt, caramel	2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (Furaneol)[d]	20	26	12	10	17	0	14	19	15 (7.8)	26
2084		animal, stable	*p*-cresol[d]	59	59	43	55	43	38	46	38	48 (8.8)	21
2128		floral, fruity	ethyl cinnamate[d]	50	24	26	38	41	31	50	33	37 (10.0)	26
2182		animal	4-ethylphenol[d]	17	30	24	41	19	24	17	19	24 (8.2)	24
2192		toasted, spicy	4,5-dimethyl-3-hydroxy-2-(5*H*)-furanone (sotolon)[d]	47	53	31	49	50	38	41	45	44 (7.2)	22
2555		vanilla, spicy	vanillin[d]	53	38	10	47	65	31	10	26	35 (19.7)	55
2633		sweet, honey	phenylacetic acid[d]	14	26	12	31	10	33	0	33	20 (12.5)	33

[a] Gas chromatographic retention data, olfactory description, chemical identity, modified frequency percentage (MF(%)), average MF(%), and standard deviation (SD, in parentheses), Max−Min MF score among wines and significance of the factor "wine". [b] Linear retention index on polar capillary column (DB-WAX). [c] Linear retention index on nonpolar capillary column (FactorFour-VF-5MS). [d] Identification based on the coincidence of gas chromatographic retention data and the similarity of odor with standars in the laboratory. [e] Not identified. The compound did not produce any clear signal in the mass spectrometer. [f] Identification based on the coincidence of gas chromatographic retention indices in both columns and mass spectrometric data with those of the pure standards available in the laboratory.

RESULTS AND DISCUSSION

Enological Parameters. Table 1 shows the enological parameters of the different wines analyzed. The parameters most influenced by sour rot infection are alcoholic degree, volatile acidity, and residual sugar content. Wines produced with damaged berries tended to have higher values of alcohol (between 12.8 and 14.6% v/v) and residual sugars (between 1.9 and 4.1 g L^{-1}), in accordance with previous results.[13] This fact is probably a consequence of berry dehydratation in rotten grapes, which leads to an increase of the fermentable sugar concentration. The volatile acidity was also systematically higher in samples from rotten grapes. In samples with 30% of damaged berries this parameter was around 0.33 g L^{-1}, a value 3 times higher than the one observed for healthy samples (around 0.12 g L^{-1}). The increase of volatile acidity can be attributed to the activity of acetic acid bacteria, the principal property of which is to oxidize ethanol into acetic acid. The capacity of the sour rot associated organisms *Acetobacter* sp., *Gluconobacter* sp., and yeast growing on rotten grapes to produce high levels of volatile acidity has been well established on the literature.[5,47] However, the higher volatile acidity of wines from rot grapes may be ascribed to the higher initial volatile acidity of musts, because the increases during fermentation were similar in all trials (results not shown).

Sensory Descriptive Analysis. The aromatic characterization of the 8 wines considered in this study was performed by a sensory panel using the 12 sensory descriptors given in Table 2. For the sake of simplicity, the results, expressed as the modified frequency (MF (%)) value, are shown separately for monovarietals of Trincadeira (Figure 1a) and for blends of Trincadeira/Cabernet Sauvignon (Figure 1b). As shown in Figure 1a, the aroma of monovarietal Trincadeira wines just after the malolactic fermentation (T0 MLF and T30 MLF) were mainly characterized by fresh fruit notes, which decreased significantly to values around 30% MF in the aged samples (T0 BOT and T30 BOT).

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This sensory pattern was not so clear for blends of Cabernet Sauvignon and Trincadeira (Figure 1b), as for these wines the fresh fruit attribute had similar values (between 45 and 55% MF) in all four samples. This could be a consequence of the vegetal/earthy character introduced by the Cabernet Sauvignon variety, which represents 70% of these blends. The attribute "chemical", which was defined as an acetic/alcoholic note, was especially high in the two samples elaborated with sour rot grapes just after the MLF, independent of the variety type (samples T30 MLF and CT30 MLF). This result can be attributed to the relatively high values of both the alcoholic degree and the volatile acidity observed in these samples (see Table 1). The honey attribute was the single one varying significantly in both sets of samples (Trincadeira and blends of Trincadeira/Cabernet Sauvignon). In both cases, the two samples elaborated from 30% infected berries presented significantly higher values of the honey attribute than blends from entirely healthy grapes. Concerning Trincadeira wines, T30 MLF and T30 BOT reached, respectively, 26 and 47% (MF) values of the honey attribute. With respect to blends of Trincadeira/Cabernet Sauvignon, CT30 MLF and CT30 BOT exhibited values around 30%. In all cases, wine samples from healthy grapes were relatively poor in the honey attribute, with MF (%) around 10. These results suggest that independently of the grape variety blend, the presence of 30% of rotten berries in the must submitted to fermentation is able to induce a honey character to the final wine. The other attribute varying significantly between samples was "floral", which presented relatively high scores in both samples of Cabernet Sauvignon and healthy Trincadeira (CT0 MLF and CT0 BOT).

Gas Chromatography−Olfactometry. The major goal of this section is to screen the odor profile of wines elaborated from healthy and from sour rotten grapes to determine the odorants that are more specific to wines elaborated with infected grapes.

The GC-O experiment was carried out on extracts obtained in a dynamic headspace system.[19] For the sake of simplicity, those odorants not reaching a maximum GC-O score (MF) of 25% in any of the eight studied wines were eliminated and considered as noise. After this operation, the number of odorants present in this set of wine extracts was 37. Table 3 summarizes the results of the GC-O study. The identification of three compounds (2-heptanol, dimethyl trisulfide, and ethyl phenylacetate) was achieved by means of heart-cutting GC techniques, which allowed us to compare the retention indices in two columns of different phases (DB-WAX and FactorFour) as well as to contrast aromatic qualities and mass spectra with those of pure standards. On the contrary, four other odorants (ni 1010, ni 1216, ni 1404, and ni 1767) could not be successfully identified even if the same heart-cutting strategy was employed.

Several odorants listed in Table 3 exhibited an average MF (%) value higher than 50: 2,3-butanedione (diacetyl); the ethyl esters of butyric, 2-methylbutyric, 3-methylbutyric, and hexanoic acids; isoamyl and β-phenylethyl alcohols; and acetic and isovaleric acids. All of the compounds in this group, for which the standard deviation (SD) of the olfactometric signal was below 10, are byproducts of alcoholic fermentation and constitute the base of wine aroma.[48] The rest of the compounds present in the table are well-known wine aroma components and have been detected in previous GC-O studies, although there are some remarkable differences between samples that are worth commenting on. For example, 3-isobutyl-2-methoxypyrazine, a well-known marker of Cabernet Sauvignon varietals,[29] and methionol were detected by the panel only in blends of Cabernet Sauvignon and Trincadeira.

Table 4. Effects of the Presence of Sour Rot in Grapes on the GC-O Aroma Profile of the Corresponding Wines[a]

	av diff[b]	t	$p(t)$
phenylacetic acid	**21.75**	**4.96**	**0.016**
ethyl hexanoate	−12	−4.83	0.017
2,3-butanedione (diacetyl)	−6.5	−4.18	0.025
ethyl 2-methylbutyrate	−6.25	−3.78	0.032
ethyl 4-methylpentanoate	−19	−3.61	0.036
***β*-phenylethyl acetate**	**7.5**	**3.38**	**0.043**
4-ethylphenol	9.25	2.66	0.076
isoamyl alcohol	−3.5	−2.65	0.077

[a] Results of a paired t test between wines from infected samples and their corresponding controls made with healthy grapes. A two-tailed t distribution with 3 degrees of freedom was used as reference distribution. [b] Average difference between the GC-O olfactometric score obtained for wines made with infected grapes and that obtained in the corresponding control.

On the contrary, the unknown compound ni 1216 with a characteristic geranium-green odor was perceived only in the extracts of monovarietals of Trincadeira, reaching a MF score near 60% in sample T0 MLF. This odorant could be successfully transferred to the second column in the GC-GC system (LRI = 914) and clearly detected by all of the panelists. Despite this, the identification could not be achieved as in this column the odorant coeluted with a major compound in wine, isoamyl alcohol, the presence of which did not allow a MS spectrum of the target compound to be obtained. This compound could be a varietal marker of Trincadeira, although it could not be detected in its blends with Cabernet Sauvignon.

To assess the effects caused by sour rot on the aromatic profile, a paired t test was carried out between infected samples and their corresponding healthy controls. The results of this test are given in Table 4. As can be seen, sour rot seems to exert a significant effect on at least eight odorants (in two cases the confidence level was <0.1). In the cases of ethyl hexanoate, diacetyl, ethyl 2-methylbutyrate, 4-methylpentanoate, and isoamyl alcohol, the presence of the rot in the grape brings about a decrease on the odorant. This result suggests a strong effect of the rot on the must amino acid profile, because, leaving aside ethyl hexanoate, the other compounds are known to be influenced by such a factor.[49,50] The likely most relevant changes from the sensory point of view are, however, the significant increments caused by rot on the GC-O levels of phenylacetic acid, β-phenylethyl acetate, and 4-ethylphenol. The increment of phenylacetic acid is particularly noteworthy. In relation to this, it is worth commenting that the ethyl ester of this acid reaches a high GC-O score on the T50 BOT sample.

Chemical Quantitative Analysis. A total of 78 major and minor volatile compounds were quantified. Results are presented in Table 5, where compounds have been arranged into 11 chemical families. Results in the table show that these wines are rather poor in varietal compounds (terpenes, norisoprenoids, volatile phenols, and vanillin and benzene derivatives) but that the wines contain surprisingly high levels of γ-decalactone. This odorant is present at levels as high as 185−405 μg L^{-1} in all of the tested wines, and its levels significantly increase ($p < 0.05$) during bottle aging, which suggests that the recently fermented wines should contain large amounts of γ-hydroxydecanoic acid from which the lactone would be slowly formed by internal esterification. To the best of our knowledge, these levels are the highest ever reported in wine, much higher than those reported

Table 5. Volatile Compound Composition of the Eight Studied Wines and Significance (p Value) of Factors "Sour Rot" (SR) and "Bottle Aging" (BA) Measured by ANOVA[a]

compound	T0 MLF	T0 BOT	T50 MLF	T50 BOT	CT0 MLF	CT0 BOT	CT30 MLF	CT30 BOT	SR effect	BA effect
carbonyl compounds										
acetoin	5254	6625	9434	9315	6958	8120	8021	8411	0.043	ns[b]
diacetyl (2,3-butanedione)	2436	2867	2563	2756	3074	3315	4025	3142	ns	ns
phenylacetaldehyde	3.12	1.57	3.06	2.56	1.79	5.78	5.75	1.80	ns	ns
esters										
ethyl butyrate	54	55	44	46	76	57	47	46	0.057	ns
ethyl hexanoate	202	183	94	78	453	154	135	122	0.096	ns
ethyl lactate	18405	65661	16442	86077	22496	83775	21916	83775	ns	0.000
ethyl octanoate	180	92	21	36	339	97	69	47	0.033	ns
ethyl decanoate	34	30	7.6	17	35	29	42	20	ns	ns
diethyl succinate	5830	12325	962	2918	20231	16438	1114	3178	0.036	ns
acetates										
isoamyl acetate	234	129	150	112	427	156	254	168	ns	ns
2-phenylethyl acetate	31	25	30	32	43	35	59	46	ns	ns
ethyl phenylacetate	nd[c]	nd	130	304	8.6	18	60	138	0.029	ns
acids										
acetic acid	167359	174938	449830	447897	134050	172290	243417	272953	0.048	ns
2-methylbutyric acid	76	42	66	31	54	64	64	68	ns	ns
3-methylbutyric acid	114	62	92	43	75	88	87	94	ns	ns
2-ethylhexanoic acid	nd	nd	nd	3.5	1.3	nd	nd	nd	ns	ns
benzoic acid	57	59	61	209	43	48	48	98	ns	ns
isobutyric acid	2252	2531	2184	2309	2210	2720	1967	2291	0.030	0.013
butyric acid	504	460	344	334	485	519	384	395	0.006	ns
isovaleric acid	2542	2510	1611	1223	2292	2862	2612	2684	ns	ns
hexanoic acid	1672	1775	766	701	1308	1852	1067	1160	0.012	ns
octanoic acid	796	1013	346	286	532	982	369	582	0.012	ns
decanoic acid	102	117	98	92	64	92	72	221	ns	ns
phenylacetic acid	102	107	1668	1557	103	85	638	645	0.041	ns
alcohols										
isobutanol	63978	64842	61147	61645	59794	59332	57702	57792	ns	ns
1-butanol	491	496	663	675	570	565	743	736	0.009	ns
isoamyl alcohol	350705	359139	202823	215213	409590	397690	341419	315347	0.075	ns
1-hexanol	507	536	441	414	847	850	748	732	ns	ns
(*Z*)-3-hexen-1-ol	2.9	2.4	3.7	2.9	7.5	8.9	10.9	9.7	ns	ns
(*E*)-2-hexen-1-ol	4.8	6.2	4.2	3.2	3.4	7.1	nd	2.6	ns	ns
benzyl alcohol	626	894	3664	3341	284	518	364	1997	ns	ns
β-phenylethanol	123291	141723	87450	62564	98309	156535	150184	134014	ns	ns
methionol	6806	8651	4441	3321	4062	7506	4586	5223	0.055	ns
terpenes										
(*Z*)-linalol oxide	nd	nd	nd	nd	nd	2.0	nd	1.9	ns	ns
(*E*)-linalol oxide	1.2	1.8	1.3	2.4	0.5	2.0	1.5	1.8	ns	0.025
linalool	nd	nd	nd	nd	nd	4.5	4.9	nd	ns	ns
α-terpineol	1.1	4.5	2.2	12	1.3	10	1.7	14.1	ns	0.005
β-citronellol	6.4	3.5	8.0	5.1	4.2	2.8	6.1	4.257	ns	0.046
geraniol	nd	nd	nd	nd	nd	nd	4.0	0.870	ns	ns
farnesol	nd	nd	nd	nd	nd	12	nd	nd	ns	ns
terpinen-4-ol	0.15	0.13	0.42	0.63	0.074	0.56	0.24	0.45	ns	ns

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Table 5. Continued

compound	T0 MLF	T0 BOT	T50 MLF	T50 BOT	CT0 MLF	CT0 BOT	CT30 MLF	CT30 BOT	SR effect	BA effect
2,6-dimethyl-1,7-octadiene-3,6-diol	nd	nd	nd	nd	nd	nd	nd	0.21	ns	ns
neric acid	6.8	6.2	6.4	6.6	3.2	3.1	3.4	3.23	ns	ns
norisoprenoids										
β-damascenone	nd	nd	nd	nd	1.23	1.06	0.54	nd	ns	ns
β-ionone	0.27	0.24	0.33	0.24	0.26	nd	0.31	nd	ns	ns
vitispirane A	0.44	1.8	0.613	2.97	0.49	2.8	0.39	2.6	ns	0.002
vitispirane B	0.71	1.3	1.09	2.48	0.67	2.6	0.82	2.8	ns	0.012
Riesling acetal	nd	nd	nd	nd	nd	2.9	nd	2.2	ns	ns
TDN	0.15	0.78	0.23	1.20	nd	0.12	nd	nd	ns	ns
TPB	nd	nd	nd	nd	nd	0.77	nd	nd	ns	ns
3-oxo-β-ionone	nd	2.8	nd	4.2	nd	7.4	nd	7.9	ns	0.019
actinidols	1.1	4.2	1.7	5.9	2.1	11	12	13	ns	ns
3-oxo-α-ionol	21	23	16	17	19	24	16	19	0.002	0.013
volatile phenols										
guaiacol	1.3	2.0	1.9	2.5	0.65	1.24	1.15	1.5	ns	ns
o-cresol	1.2	1.1	1.1	1.3	0.99	1.08	1.09	1.0	ns	ns
4-ethylguaiacol	nd	nd	0.35	0.52	1.78	1.95	2.38	0.31	ns	ns
eugenol	2.41	2.53	2.94	3.59	0.81	1.17	1.3	1.75	ns	ns
4-ethylphenol	0.46	0.45	0.67	0.74	0.53	0.56	0.45	0.47	ns	ns
4-vinylguaiacol	3.9	6.2	3.9	6.6	3.4	3.6	4.5	4.6	ns	ns
2,6-dimethoxyphenol	8.9	20	7.9	15	4.9	14	4.9	11	ns	0.016
(*E*)-isoeugenol	2.3	1.8	4.0	3.1	1.5	0.82	2.9	1.9	ns	ns
4-vinylphenol	40	143	13	33	13	39	7.6	18	ns	ns
vanillin derivatives										
vanillin	5.6	13	5.3	12	3.6	12	2.8	9.2	ns	0.003
methyl vanillate	17	18	18	21	17	17	18	14	ns	ns
ethyl vanillate	167	354	256	596	95	162	109	197	ns	ns
acetovanillone	79	81	76	78	32	34	29	32	ns	ns
zingerone	26	28	19	14	nd	7.4	6.9	6.8	ns	ns
homovanillic acid	32	nd	nd	nd	nd	52	34	8.7	ns	ns
homovanillyl alcohol	3.5	nd	nd	nd	nd	nd	nd	nd	ns	ns
benzene derivatives										
benzaldehyde	2.3	4.1	29	25	2.5	3.5	8.1	7.8	ns	ns
2-phenoxyethanol	0.99	0.78	1.47	1.12	1.10	1.38	0.91	nd	ns	ns
dihydromethyleugenol	14	15	21	22	10	11	13	14	ns	ns
ethyl dihydrocinnamate	nd	nd	nd	nd	nd	0.085	0.19	0.21	ns	ns
ethyl cinnamate	0.81	0.82	0.83	1.3	0.65	0.68	0.50	0.64	ns	ns
lactones										
δ-octalactone	4.04	3.2	8.8	8.9	2.5	2.9	4.3	4.9	ns	ns
γ-nonalactone	10	9.7	37	37	3.2	4.0	16	19	0.049	ns
γ-decalactone	203	302	280	405	202	297	185	319	ns	0.025
δ-decalactone	5.0	8.1	4.7	8.8	3.7	5.7	3.7	4.5	ns	ns
γ-butyrolactone	5802	12232	12223	14932	5040	8947	7475	10701	ns	ns

[a] Compounds showing significance ($p < 0.1$) for any of the factors are highlighted in bold. Quantitative data are expressed as μg L^{-1}. [b] ns, not significant. [c] nd, not detected.

in young[29] or aged[51] Spanish reds, Australian reds,[52] or even Uruguaian tannats,[18] for which maxima levels of 75 μg L^{-1} were found. For this compound thresholds of 88 μg L^{-1} [53] and 790 μg L^{-1} [51] have been reported, and a synergic action with the other

Table 6. Effects of the Presence of Sour Rot in Grapes on the Levels of Volatile Compounds of the Corresponding Wines[a]

compound	av diff[b]	inc[c] (%)	p(SR)
3-oxo-α-ionol	−4.75	−21.8	0.002
butyric acid	−128	−26.0	0.006
1-butanol	**174**	**32.8**	**0.009**
hexanoic acid	−728	−44.1	0.012
octanoic acid	−435	−52.4	0.012
ethyl phenylacetate	**149**	**1627**	**0.029**
isobutyric acid	−240	−9.9	0.030
ethyl octanoate	−134	−75.6	0.033
diethyl succinate	−11663	−85.1	0.036
phenylacetic acid	**1028**	**1035**	**0.041**
acetoin	**2056**	**30.5**	**0.043**
acetic acid	**191365**	**118**	**0.048**
γ-nonalactone	**20.5**	**305**	**0.049**
methionol	−2363	−35.0	0.055
ethyl butyrate	−14.7	−24.4	0.057
isoamyl alcohol	−110580	−29.2	0.075
ethyl hexanoate	−141	−56.8	0.096

[a] Results of ANOVA test between wines from infected samples and their corresponding controls made with healthy grapes. [b] Average difference between the levels (in $\mu g\ L^{-1}$) obtained in wines made with infected grapes and those obtained in the corresponding controls. [c] Increase (%) with respect to the average content of the compound in the control wines.

γ-lactones has also been found,[51] which would indicate that γ-decalactone can be an important aroma compound in Trincadeira wines.

Results of the ANOVA tests carried out on those data to assess the effects of sour rot are summarized in Table 6, which mostly confirm GC-O observations. As shown in the table, the presence of sour rotten grapes exerts a deep effect on the levels of fatty acids and their ethyl esters formed by yeast, which decreased nearly by a 2 factor on average. The levels of some volatiles related to the synthesis and catabolism of amino acids, such as isobutyric acid, methionol, and isoamyl alcohol, were also significantly reduced. These changes suggest that the presence of sour rot exerts a deep impact on the secondary metabolism of yeast with a likely relevant impact on the wine sensory properties. It is remarkable that only one purely varietal compound, the norisoprenoid 3-oxo-α-ionol, was significantly affected by sour rot.

On the other hand, six compounds (1-butanol, ethyl phenylacetate (EPhA), phenylacetic acid (PAA), acetoin, acetic acid, and γ-nonalactone) present significantly higher concentrations ($p < 0.05$) in wines made from sour rotten grapes. As seen in the table, increases in the cases of 1-butanol and acetoin are not very large, quantitatively, and most likely will not cause any relevant sensory impact. In the case of acetic acid, the increment is relatively important in quantitative terms (118% in average), although the final levels on the wines still can be considered normal. In the case of γ-nonalactone, the increments are really notable, and the levels finally produced in the T50 samples are above the 30 $\mu g\ L^{-1}$ threshold for this compound.[54] However, the highest effects are noted on the levels of EPhA and PAA, which increase by factors on average above 1 magnitude of order as a consequence of the presence of sour rot. In the case of monovarietals of Trincadeira (50% of sour rotten grapes), the concentration of EPhA in sour rotten samples reached 130 and 304 μgL^{-1} in wines after malolactic fermentation and bottle aging, respectively. Blends of Trincadeira/Cabernet Sauvignon exhibited slightly smaller concentration levels (60 and 138 μgL^{-1}), as only 30% of sour rotten grapes were employed. The same pattern was observed for PAA ($p < 0.05$), the direct precursor from which the acetate is formed by esterification.

In both set of wines, the concentrations of both EPhA and PAA in sour rotten wines were above their corresponding odor thresholds: 73 $\mu g\ L^{-1}$ for the ester[55] and 1000 $\mu g\ L^{-1}$ for the acid.[56] Considering that these two compounds are associated with a clear honey-like and sweet aroma, EPhA and PAA could be considered as responsible of the honey-sweet aroma associated with wines elaborated from sour rot infected grapes.

PAA has already been identified as the main odorant in sweet Fiano wines,[57] which are produced with grapes in an advanced state of maturation and dried on racks for sugar concentration. Genovese et al.[41] found 181 $\mu g\ L^{-1}$ of EPhA in those Fiano wines. More recently, Tat et al.[55] attributed the untypical "sweet-like" off-odor developed in some samples (empirically connected with some cryptogamic diseases) of Italian Aglianico del Vulture wine, also produced with a late-ripening variety, to this compound. It should be noted that the maximum levels of EPhA in those defective samples were 150 $\mu g\ L^{-1}$, a figure well below the 304 $\mu g\ L^{-1}$ found in our T50 BOT sample.

The biosynthetic pathways that lead to the formation of EPhA in wines are not clearly understood, although this compound could be produced by esterification of PAA during alcoholic fermentation.[55] PAA is known for its weak auxin activity in a range of crop plants,[58] working as a plant growth regulator, and may be involved in plant defense mechanisms.[59] Moreover, Ziauddin et al.[60] showed a striking response in plant regeneration from wheat anther and on barley anther/microspore culture using PAA in the induction medium. In light of these studies, our results, and the empirical observation that the honey-like smell is not present in Aglianico wines produced without skin contact,[55] our hypothesis is that an alteration of berry surface could activate a plant response mechanism that leads to the production of high concentrations of PAA. This compound could be extracted to the must if a maceration process occurs and be further converted to the ester (EPhA) during fermentation or later during bottle aging.

In summary, this study shows that sour rot infection clearly affects both the chemical and the sensory profiles of wines elaborated with blends containing between 30 and 50% of damaged grapes. Our research strongly indicates that EPhA and PAA may be taken as chemical markers of sour rot infection in grapes and that both compounds are most likely responsible for the characteristic honey note evoked by these products. Sour rot also increases the levels of γ-nonalactone in wine. Results also suggest that sour rot exerts a great effect on the secondary metabolism of yeast, decreasing the levels of volatiles related to fatty acids and amino acid synthesis. Finally, the high levels of γ-decalactone found in all of the samples suggest that this compound may be a relevant odorant of Trincadeira wines.

More research is needed to understand the biochemical pathways that lead to the synthesis of PAA and EPhA in damaged berries.

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