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Changes in Wine Aroma Composition According to Botrytized Berry Percentage: A Preliminary Study on Amarone Wine

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Summary

The aim of this study is to evaluate the impact of *Botrytis cinerea*, a noble rot, on the aroma components of Amarone, a dry red wine produced from withered grapes. A comparative analysis of wines obtained from manually selected healthy and botrytized grapes was done. Aroma analysis revealed that most compounds varied significantly according to the percentage of botrytized berries utilized. Botrytized wines contained less fatty acids and more fruity acetates than healthy wines. A positive correlation between the content of *N*-(3-methylbutyl)acetamide, sherry lactone and an unidentified compound and the level of fungal infection was also observed. The results indicate that noble rot can significantly modify important aroma components of Amarone wine.

Key words: noble rot, *Botrytis cinerea*, botrytized wine, wine aroma, Amarone wine

Introduction

Amarone is a passito red wine produced from partially dried grapes of Corvina and Rondinella varieties in Verona area (Italy). This wine is dry because the devatting is carried out when the alcoholic fermentation is completed. After two years of ageing, Amarone wine can be commercialized. Ripe fruits, prune, cherry jam, toasted almond, licorice, canella and spicy are its main aromatic descriptors.

The grape withering process can last up to three months, allowing partial water removal with a mass loss up to 40 % (*1*). During this process the grapes are susceptible to *Botrytis cinerea* infection that, at the stage of 'grey rot', may induce negative and undesirable modification of wine (*2*). On the contrary, *B. cinerea* under climate conditions with alternating brief damp and longer dry periods (*3*) can develop as a noble rot giving a positive impact on the overall quality of certain white wines (called 'botrytized wines'), such as Sauternes and Tokaji Aszú (*2,3*). In the case of Amarone wine the withering of grapes can occur under natural conditions or in a conditioned chamber with forced ventilation and strictly controlled relative humidity and temperature. In the latter case, the grape dehydration rate is optimized and they are less susceptible to mould infection (*1*). In natural grape withering, the producers periodically check the mould infection manually, discarding the berries and grape bunches affected by *B. cinerea*. To date, a significant production of Amarone wine is still handicraft, obtained by fermentation of naturally withered grapes, and the influence of noble rot on its chemical and sensorial profile is quite unknown.

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The improvement of noble rot development on harvested grapes is also a production strategy for botrytized wines in several countries with unsuitable climates, such as California and Australia. Botrytized wines are renowned for a wide range of aromas, evoking citrus, dried fruits and honey. These sensorial evolutions are consequences of important transformations of the aroma compounds occurring in the must and during the fermentation of infected grapes. The contribution of noble rot to the volatile aroma composition of botrytized wines is well documented (*4–8*). Precursors of some varietal compounds (terpenes and C13 norisoprenoides) are originated by metabolic activity of the fungus (*9–11*). The content of fermentative acids and esters can vary significantly in relation to the level of *Botrytis* infection (*2,12*). In Tokaji Aszú made from botrytized grapes, the concentration of hydroxy-, oxo- and dicarboxylic acid esters is higher than in other wine types (*6,7*). Moreover, it has been demonstrated that volatile thiols have a huge impact on botrytized wine aroma (*8,13–15*).

Most of the investigations regarding the role of noble rot in the wine aroma was carried out on sweet white wines, such as Sauternes and Tokaji Aszú, but little information on Amarone wine is available. The aim of this study is to evaluate the effect of grape noble rot infection on the aroma compounds of this passito wine. A comparative analysis was done between wines obtained from the healthy and the mixed healthy and botrytized grapes.

Materials and Methods

Experimental design and microvinifications

Amarone wine was produced from grapes of the Corvina and Rondinella varieties, according to its production regulations. The grapes were partially dried under natural conditions for about 4 months using the traditional overripenining (surmaturation) technique in the Valpolicella area (Verona, Italy). Berries were visually selected on the basis of the absence or presence of *B. cinerea* according to Delfini (*16*). Those infected with grey rot, less than approx. 5 % of the total, were discarded. Berries unaffected and affected by noble rot were separated manually into two batches, healthy (H) and botrytized (B) ones, respectively. The batches were crushed separately and, in order to obtain homogenous trials for the red vinifications, the liquid (must) and solid (grape pomace) fractions were separated, mixed and subsequently divided maintaining their proportion. The resulting B and H musts were used to obtain three different wines. W-100 (healthy wine) was produced only from H must, while W-80 and W-60 (botrytized wines) were produced mixing together 80 % of H and 20 % of B musts and 60 % of H and 40 % of B musts, respectively. The final volume of wines was 10 L. Each wine was produced in triplicate, undergoing separate vinifications.

Before the use of commercial yeast starter, *Saccharomyces cerevisiae* EC1118 (Lallemand Inc, Montréal, Canada), 50 mg/L of SO_2 were added. The trials were conducted in a local winery without controlled temperature, which ranged between 11 and 15 °C during the experimentation.

The alcoholic fermentation (AF) was monitored by ethanol production and sugar consumption and it was considered terminated when the content of reducing sugars was less than 3.0 g/L. After AF, the wines were devatted and decanted at 10 °C for 4 days, then stabilized by adding 50 mg/L of SO_2 before the analysis. The wine did not undergo malolactic fermentation. This fermentation can occur when the conditions for the growth of malolactic bacteria are favourable, but often the presence of high ethanol content (>14.0 % by volume) hinders the transformation of L-malic to L-lactic acid. Then Amarone wine is commercialized irrespective of the completion of malolactic fermentation (data not published).

Wine analysis

Ethanol was analyzed by NIR spectroscopy using Alcolyzer Wine apparatus (Anton Paar GmbH, Graz, Austria). Glucose and fructose, organic acids and glycerol were quantified using enzyme kits (Hoffmann-La Roche, Basel, Switzerland).

Volatiles were analyzed by gas chromatography-mass spectrometry (GC-MS) after solid-phase extraction (SPE) and headspace solid-phase microextraction (HS-SPME). After SPE using ENV^+ cartridge (1 g, 40–140 μm; Isolute, IST Ltd., Mid Glamorgan, UK), aroma compounds were analyzed by GC-MS (*17*). The process was performed by an Aspec XL Sample Processor for SPE (Gilson Inc., Middleton, WI, USA). The cartridges were sequentially conditioned with methanol (10 mL) and distilled water (10 mL). A total of 76 mL of wine sample diluted 1:4 (by volume) with distilled water, and 1-heptanol added as internal standard (500 μg/L) was loaded onto the cartridge. The residue was washed with 10 mL of distilled water. The free aroma compounds were eluted with 9 mL of dichloromethane. The solution was dried with Na_2SO_4 and concentrated to 0.4 mL by nitrogen flow stream.

GC-MS analysis was performed with a 6890N Network GC System coupled with a 5978B inert XL EI/CI MS (Agilent Technologies, Santa Clara, CA, USA), equipped with an HP-WAX Bonded PEG fused silica capillary column (60 m×320 μm i.d.×0.25 μm film thickness; Agilent Technologies). Instrumental conditions were: electron impact (EI) mode 70 eV; injector temperature 250 °C; He carrier flow 1.5 mL/min; column temperature 50 °C for 4 min, rising to 240 °C at 4 °C/min, then 16 min at 240 °C; and injection volume 0.4 μL in splitless mode.

In order to improve the information about the volatile compounds affected by the presence of *B. cinerea* infection on the grapes, a HS-SPME procedure was adopted. The analysis was carried out according to Versini *et al.* (*18*) using methyl heptanoate as internal standard (final concentration in 5 mL of wine equal to 100 μg/L). The same GC-MS instrument adapted for HS-SPME analysis through a fused silica fibre DVB/CAR/PDMS (2 cm×30/50 μm; cod. no. 57348-U, Stable Flex, Supelco Inc., Bellefonte, PA, USA) was used. The extracted compounds were thermally desorbed in the GC injector for 5 min (injector maintained at 250 °C) and transferred into the column by inserting the fibre into the GC injector port at 50 °C for 5 min, rising to 230 °C at 12 °C/min, then at 230 °C for 10 min. Six calibration levels were prepared and every level was analyzed in triplicate. Table 1 reports the statistical parameters of the calibration curves.

Table 1. Calibration parameters and detection limits of each compound analysed by SPME

Analyte	Slope	SD slope	Intercept	SD intercept	SD	R	γ(LD)/(μg/L)
p-cymene	1.21	0.01	0.011	0.008	0.04	0.992	0.56
γ-terpinene	1.14	0.04	0.014	0.013	0.04	0.993	0.23
α-terpinolene	3.25	0.02	0.024	0.008	0.03	0.994	0.11
linalylethyl ether	1.25	0.02	0.021	0.014	0.08	0.998	0.41
α-terpenylethyl ether	1.03	0.03	0.010	0.022	0.01	0.993	0.26
nerylethyl ether	1.43	0.01	0.012	0.013	0.15	0.992	0.31
geranylethyl ether	1.21	0.01	0.014	0.008	0.03	0.994	0.47
TDN	1.27	0.02	0.012	0.014	0.20	0.995	0.77
vitispiranes	2.23	0.08	0.006	0.037	0.05	0.991	0.61
furfural	1.35	0.04	0.014	0.026	0.07	0.995	1.65
styrene	1.29	0.03	0.013	0.032	0.11	0.997	1.54

SD=standard deviation, R=coefficient of linearity, LD=detection limits

All the analyses, both SPE and HS-SPME, were performed in SCAN mode. NIST data bank and co-injection of pure reference standards were used to identify the compounds.

Statistical treatment of the data

The values of wine compounds reported in the text are average (±standard deviation) of three independent samples (replications), one for each vinification. The *t*-test was performed for each wine compound to test the differences in the wine samples.

Results and Discussion

The content of ethanol and acetic acid of the experimental wines ranged between 14.3 and 14.9 % by volume and 0.12 and 0.25 g/L, respectively. The content of reducing sugars was less than 3.0 g/L in all wines (2.7, 2.3 and 2.2 g/L of glucose and fructose in W-100, W-80 and W-60, respectively). These parameters are considered as standard for Amarone wine according to its production regulation. B wines contained more glycerol and gluconic acid than H wine (data not shown) in accordance with the percentage of infected grapes by *B. cinerea*. Glycerol and gluconic acid ratio represents a noble rot quality index, and a high glycerol/gluconic acid ratio indicates the *pourri plein* stage predominance (*3*,*19*).

The results of the analysis of aroma compounds performed in H and B wines are reported in Table 2. Thirty-seven out of 64 aroma compounds differed in their concentration significantly. In particular, 24 out of 37 aroma compounds varied more than 30 % in W-80 and/or W-60 with respect to W-100 (in bold in Tables 2 and 3).

In relation to C6 alcohols (Table 2), an increase of *trans*- and *cis*-3-hexenol was found in B wines (green grass/chocolate note associated with 1-hexanol), although their concentrations were quite low with respect to those measured in typical red wines (*20*,*21*). No significant changes in the amount of aromatic alcohols (benzyl alcohol and 2-phenylethanol) were observed among the three wines.

The 1-octen-3-ol (mushroom note) is an off-flavour that is often detected in wines produced from grapes affected by grey rot and has an olfactory perception threshold of 25 μg/L according to La Guerche *et al.* (*22*). This compound can also be detected in botrytized wines, but it is found below its perception threshold, as in the case of B wines (Table 2).

Tendency to a limited increase of isoamyl and 2-phenethyl acetate (ripe fruit note) (Table 2) with the grape botrytisation level was observed in B wines, even if resulting in a quite low level, as typical for aged red wines. Ethyl esters (C6–C10), contributing to the fruity scent (*23*), and their related fatty acids (powdery/greasy note) were higher in H wine. Previous investigations reported the decrease of the content of isoamyl, 2-phenethyl acetate and ethyl esters (C6–C10) in botrytized wines due to the esterase activity of *B. cinerea*, which persists in the juice, especially when grapes are infected by grey rot (*2*,*12*). It is probable that in B wine (obtained from grape infected by noble rot) this activity was not so high to determine the decrease of isoamyl and 2-phenethyl acetate content. The different amount of exocellular hydrolytic enzymes in the juices, depending on the level of grape infection, could influence significantly the content of these esters. Moreover, variation in the production of these volatile compounds could depend on yeast strains that conducted AF and on their different ability to metabolize nitrogen (*24*). The diversity of ethyl esters observed in Amarone wines could also be due to different composition of fermentative substrates that depends on the level of *Botrytis* infection. Skin of the infected berries is more breakable than that of healthy berries and this determines the different content of solid particles in contact with grape juice (*25*). In Aszú wines, different content of ethyl hydroxyesters and dicarboxylic esters was related to the *Botrytis* infection (*4*,*7*), confirming what was observed in Amarone wines (as a matter of fact, higher content of these compounds was found in B wine) (*7*). The oxidative metabolism of *B. cinerea*, prevailing in the injured berry skins, plays an important role in the generation of these volatile fermentation products besides other aroma-characteristic compounds of botrytized wines (*5*).

The increase of ethyl phenylacetate (Table 2), depending on the percentage of botrytized grapes, is also interesting. This compound is mainly responsible for the

Table 2. Aroma compounds detected by SPE analysis in wines obtained from the fermentation of the must from healthy grapes (W-100) and musts from mixed healthy and botrytized grapes at different percentages (W-80 and W-60)

Aroma compound	γ/(μg/L)		
	W-100	W-80	W-60
C6 alcohols			
1-hexanol	(1580±71)^{a}	(1759±32)^{b}	(1594±119)^{ab}
***trans*-3-hexenol**	(23±2)^{a}	(28.0±0.9)^{b}	(30±2)^{b}
cis-3-hexenol	(53±4)^{a}	(68±8)^{ab}	(67±7)^{b}
Subtotal	(1656±76)^{a}	(1855±33)^{b}	(1691±114)^{ab}
Other alcohols			
benzyl alcohol	437±18	417±39	391±51
2-phenylethanol	13355±1873	13317±623	12841±377
methionol	(176±15)^{a}	(483±116)^{b}	(345±87)^{ab}
1-octen-3-ol	(9±3)^{a}	(15±1)^{b}	(16±2)^{b}
Subtotal	13978±1845	14233±522	13593±366
Fermentative esters			
isoamyl acetate	(16±3)^{a}	(30±5)^{b}	(36±2)^{b}
2-phenylethyl acetate	(32±2)^{a}	(42±4)^{b}	(50±5)^{b}
ethyl hexanoate	(489±20)^{a}	(425±65)^{ab}	(385±11)^{b}
ethyl octanoate	360±44	330±67	268±57
ethyl decanoate	75±16	74±16	58±13
ethyl 2-hydroxy-isovalerate	(23±3)^{a}	(35±2)^{b}	(40±2)^{b}
ethyl 3-hydroxybutyrate	514±18	507±52	493±7
ethyl 2-hydroxy-4--methylpentanoate	(115±4)^{a}	(161±11)^{b}	(179±11)^{b}
ethyl 4-hydroxybutyrate	573±107	712±184	813±136
diethyl 2-hydroxyglutarate	(4899±447)^{a}	(6044±511)^{b}	(6227±780)^{ab}
ethyl phenylacetate	(5.2±0.6)^{a}	(12±2)^{b}	(20±2)^{c}
Subtotal	7099±309	8370±913	8569±929
Fatty acids			
butyric acid	735±72	732±43	666±44
isovaleric acid	258±35	305±20	311±70
hexanoic acid	(1546±11)^{a}	(1328±28)^{b}	(1207±108)^{b}
octanoic acid	(1557±75)^{a}	(1304±20)^{b}	(1191±203)^{ab}
decanoic acid	326±33	316±14	288±51
Subtotal	(4422±144)^{a}	(3985±67)^{b}	(3663±456)^{ab}
Aldehydes and ketones			
vanillin	11±2	11±3	10±2
benzaldehyde	(12±3)^{a}	(17±4)^{a}	(21±4)^{b}
phenylacetaldehyde	(0.2±0.1)^{a}	(2.9±0.7)^{b}	(2.8±0.6)^{b}
norfuraneol	(56±6)^{a}	(79±5)^{b}	(81±12)^{ab}
Subtotal	(79±6)^{a}	(110±10)^{b}	(114±8)^{b}

Aroma compound	γ/(μg/L)		
	W-100	W-80	W-60
Terpenes			
***trans*-furanic linalool oxide**	(5.0±0.3)^{a}	(6.4±0.7)^{ab}	(7.2±0.4)^{b}
***cis*-furanic linalool oxide**	(4.1±0.4)^{a}	(5.5±0.4)^{b}	(6±1)^{ab}
trans-pyranic linalool oxide	3.1±0.4	3.2±0.1	3.9±0.4
cis-pyranic linalool oxide	(1.4±0.0)^{a}	(1.5±0.2)^{ab}	(1.8±0.1)^{b}
linalool	2.4±0.1	2.5±0.4	2.6±0.5
α-terpineol	(14.4±0.9)^{a}	(18.3±0.8)^{b}	(15±1)^{a}
4-terpinenol	(10±2)^{a}	(13±1)^{a}	(18±2)^{b}
ho-diendiol I	(3.9±0.3)^{a}	(4.6±0.1)^{b}	(4.5±0.4)^{a}
ho-diendiol II	2.2±0.4	2.9±0.3	3.3±0.6
Subtotal	(47±1)^{a}	(58±4)^{b}	(61±2)^{b}
C13-norisoprenoides			
β-damascenone	2.6±0.6	2.8±0.6	2.9±0.4
3-oxo-α-ionol	(31.6±0.5)^{a}	(39±1)^{b}	(40±2)^{b}
Subtotal	(34±1)^{a}	(42±2)^{b}	(43±2)^{b}
Lactones			
sherry lactone (isomer 2)	(215±10)^{a}	(311±33)^{b}	(386±32)^{c}
sherry lactone (isomer 1)	1902±121	1890±246	1957±103
γ-nonalactone	6.8±0.8	8.0±0.2	10±2
4-carboxyethoxy-γ-butyrolactone	2279±38	2671±263	2713±374
Subtotal	4403±146	4880±276	5066±461
Benzenoids			
methyl vanillate	(10±1)^{a}	(13±1)^{b}	(13.9±0.3)^{b}
ethyl vanillate	(78±6)^{a}	(112±25)^{ab}	(127±12)^{b}
acetovanillone	(113±5)^{a}	(132±16)^{ab}	(142±5)^{b}
homovanillic alcohol	122±4	132±7	124±9
homovanillic acid	(11.3±0.5)^{a}	(14±1)^{b}	(15.7±0.6)^{b}
vanillic acid	146±28	98±11	176±41
Subtotal	(480±38)^{a}	(501±37)^{a}	(599±41)^{b}
Phenols			
phenol	(4.1±0.3)^{a}	(4.6±0.3)^{ab}	(5.1±0.4)^{b}
o-cresol	(1.6±0.1)^{a}	(1.7±0.3)^{ab}	(1.8±0.0)^{b}
p-cresol	1.3±0.1	1.0±0.0	1.2±0.2
Subtotal	7.0±0.3	7.2±0.6	8.2±0.6

Table 2. – continued

Aroma compound	γ/(μg/L)		
	W-100	W-80	W-60
Other			
***N*-(3-methylbutyl)-acetamide**	(65±3)[a]	(915±142)[b]	(1878±138)[c]
not identified	(1995±179)[a]	(2598±369)[ab]	(3287±80)[b]
Subtotal	(2060±182)[a]	(3513±377)[b]	(5165±96)[c]
Total	(34265±1865)[a]	(37554±1629)[ab]	(38563±550)[b]

Mean values with different letters in superscript within a row are significantly different ($p<0.05$);

The compounds in bold whose concentrations varied statistically more than 30 % in W-80 and/ or W-60 with respect to W-100

Table 3. Aroma compounds detected by SPME analysis in wines obtained from the fermentation of the must from healthy grapes (W-100) and the must from mixed healthy and botrytized grapes at different percentages (W-80 and W-60)

Aroma compound	γ/(μg/L)		
	W-100	W-80	W-60
***p*-cymene**	(21±1)[a]	(25±5)[a]	(39±2)[b]
γ-terpinene	(20±4)[a]	(29±5)[ab]	(33±5)[b]
α-terpinolene	2.9±0.1	2.9±0.4	2.9±0.7
linalylethyl ether	12±1	10±3	10±2
α-terpenylethyl ether	35±6	57±14	45±9
nerylethyl ether	8±1	6±21	7±2
geranylethyl ether	2.6±0.5	4.6±0.6	4.2±0.8
TDN	(12.3±0.9)[a]	(9±2)[ab]	(6±2)[b]
vitispiranes	8±2	11±1	12±1
furfural	(21±2)[a]	(34±9)[ab]	(51±6)[b]
styrene	14±1	17±3	16±2
Total	(159±15)[a]	(204±8)[b]	(226±12)[b]

Mean values with different letters in superscript within a row are significantly different ($p<0.05$)

The compounds in bold whose concentrations varied statistically more than 30 % in W-80 and/or W-60 with respect to W-100

sweet-like off flavour of Aglianico del Vulture wine, a red bodied wine that ages similarly to Amarone wine (*26*).

The analysis of phenylacetaldehyde (note of flower and honey-hyacinth) and benzaldehyde (bitter almond note) (Table 2) confirmed their association with the presence of *B. cinerea* on grapes (*8,18,27,28*). In fact, B wines contained higher level of these two compounds with respect to H wine. The increase of benzaldehyde in botrytized wines is mainly attributed to the ability of *B. cinerea* to convert benzyl alcohol (*27,29*).

The content of furfural (Table 3) differed between H and B wine, as reported in Fiano wine analysis (*30*), attesting to higher levels than those found in red wines by Escudero *et al.* (*21*). The oxidation of wine influences greatly the level of this aldehyde (*31*), but the relationship between the furfural formation and mould infection is still not clear. In fact, this compound can be derived from carbohydrate dehydration followed by Maillard reaction and, to date, its generation by enzymatic conversion of pentosans has not been demonstrated.

Specific chemical descriptors of botrytized wines (Sauternes and Tokaji) such as furaneol, homofuraneol and norfuraneol had previously been analyzed (*8*). The third descriptor was the most abundant in Amarone wine (Table 2) and it increased in B wine, while the other two descriptors were detected in a concentration below 5 and 2 μg/L, respectively (data not shown).

No substantial differences related to the content of monoterpenes of varietal origin among the Amarone wines were observed (Tables 2 and 3). The variation of these compounds, due to *B. cinerea* grape infection, was analyzed mainly in Muscat varieties, which are characterized by high content of terpenes (*9,11,19*). A limited increase of furanic linalool oxides and *cis*-pyranic linalool oxides as well as ho-diendiol I (2,6-dimethyl-3,7-octadiene-2,6-diol) was observed in B wine, which is in agreement with Rapp *et al.* (*11*). The increase of linalool oxides could be due to the chemical oxidation and also to its biotransformation by *B. cinerea*. Also, 4-terpineol can be transformed in other terpenic compounds (*10*). Its content in W-60 was higher than in the other Amarone wines, suggesting a positive correlation between this monoterpenoid and the presence of *B. cinerea* in the grapes. Nevertheless, 4-terpineol concentration detected in these wines was rather low with respect to that detected in other passito Italian wines (*30,32*). Higher content of *p*-cymene found in B wines is in accordance with Genovese *et al.* (*30*), who detected this terpene only in Fiano wine obtained from botrytized grapes. The level of γ-terpinene, higher in B wines than in H wine, was low with respect to that reported by Piñeiro *et al.* (*33*) in different red wines.

Besides the role of *B. cinerea* metabolism, described above, the occurrence of specific metabolic activities of other fungi, harbouring the infected grapes, can favour the variation in the content of the monoterpenes. Arévalo Villena *et al.* (*34*) investigated the release of glucoside terpenes by specific glucosidase contained in yeasts forming part of wine ecosystem that enhances the grape varietal characteristics. Monoterpenes such as citronellol, nerol and geraniol were, however, detected at a concentration lower than 2 μg/L in all Amarone wines (data not shown).

In relation to norisoprenoides, the increase of 3-oxo-α-ionol (tobacco note) (Table 2) can be associated with the fungal glycosidase, which can hydrolyze not only terpene glycosides but also C13 norisoprenoids (*35*) in B wine. In fact, the generation of these compounds is mainly regulated by enzymatic cleavage rather than chemical and oxidase-coupled degradation.

The amount of four lactones analyzed in Amarone wines was related to *B. cinerea* infection according to previous studies carried out on Tokaji Aszú wines (*6,7*). B wines showed a higher content of one out of two iso-

mers (the one with shorter retention time) of sherry lactones (Table 2), typical compounds found in oxidized wines like Sherry wine (*36*,*37*). This isomer is most likely the lactone of (4*R*,5*R* or 4*S*,5*S*)-4,5-dihydroxyhexanoic acid (*38*). Among alkyl lactones, only γ–nonalactone (coconut note) (Table 2) slightly increased in B wines, remaining at a low level with respect to that found in commercial botrytized wine (*39*).

Benzenoids increased in B wines, in particular methyl and ethyl vanillate, acetovanillone and homovanillic acid (Table 2). These are mainly varietal compounds obtained by the hydrolysis of the bound forms (*40*). The rather high acetovanillone concentration could be related to a honey-like attribute in red wines, according to Francis *et al.* (*41*).

Clear increase of *N*-(3-methylbutyl)acetamide (vinegar note) and methionol (cooked potato note) (Table 2) was observed in B wines. Such increase had previously been described in white wine produced by grapes harvested early or by maceration process (*38*).

Finally, a strong increase of an unidentified compound, probably a derivative of a hydroxyacid with empirical formula $C_5H_8O_5$ (M_r=148) (Table 2), was detected in B wines. Its retention time was close to that of 2-phenethyl acetate with the main MS fragments of 76 (100), 59 (76), 104 (57), 43 (41), 31 (27), 89 (27), 71 (27), 147 (17), 119 (7) and 148 (<1). This compound had previously been recognized by Di Stefano (*42*) in aged Riesling wine. In particular, the fragments 76 (100) and 59 (76) could correspond to compounds with empirical formula $C_2H_4O_3^+$ and $C_2H_3O_2^+$, respectively.

Conclusions

In conclusion, the results reported here show a significant impact of *Botrytis cinerea* infection, a noble rot, on the aroma compounds of Amarone wine. Botrytized wines contained fewer ethyl esters (C6–C10) and related fatty acids as well as more fruity acetates, such as isoamyl and 2-phenethyl acetate, than healthy wines. Besides the detection of typical analytical descriptors, such as 4-terpineol, benzaldehyde, phenylacetaldehyde and norfuraneol, other molecules such as *N*-(3-methylbutyl)-acetamide, sherry lactones and an unidentified compound (M_r=148) seem to be interesting for analysis although their genesis is nowadays unknown. Nevertheless, further compositional and sensory analyses are needed to study the effect of *B. cinerea* on wine aroma in more details.

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