

# Botrytized wines – current perspectives

Ildikó Magyar  
János Soós

Department of Oenology, Institute of  
Viticulture and Oenology, Szent István  
University, Budapest, Hungary

**Abstract:** Botrytized wines are wine specialties made of overripe grapes infected by *Botrytis cinerea* with the form “noble rot”. Due to the particular characteristics of the noble rotted grape, these wines (eg, Tokaji Aszú, Sauternes, Trockenbeerenauslese types, etc) have many characteristic features, including higher or lower residual sugar content and unique aroma composition. The technology, biochemistry, and special characteristics of botrytized wines have been researched for a long time. This review outlines the main directions of the current studies, giving a brief overview on the recent findings. Beside the traditional wine types, noble rot is increasingly utilized in making newer sweet wine styles and straw (passito) wines, which generates a series of new interesting experimental results. The fungus–grape interactions during the noble rot, the induced botrytization, the microbial communities of botrytized wines, and the volatile compounds having key roles in the distinct aroma of these wine styles are being focused on in the current studies in this field.

**Keywords:** *Botrytis*, noble rot, sweet wines, passito wine, aroma

## Introduction

Botrytized wines are a distinct category of the natural sweet wines produced from withered, shriveled grapes. While the other sweet styles such as late harvest wines, ice wines, and traditional straw (“passito”) wines are mostly made of physically dehydrated, concentrated grape juices, botrytized wines are produced from noble rotted grape, which is not only dehydrated but also infected and transformed by *Botrytis cinerea* under special conditions. In contrast to the usual detrimental activity of this fungus on grapes (gray rot), noble rot is a rare, desirable process, which greatly enhances grape quality. During noble rot, some of the compounds of the grape are decomposed, while new compounds are produced by the fungus. This modified chemical composition of the grape juice is highly concentrated due to the physical effect of dehydration, which is supported by the digested grape skin and by particular weather conditions (morning dust/fog and dry, sunny days). The wines produced from this extremely high sugar and extract content grape have many characteristic features, including higher or lower residual sugar content and unique aroma composition. Some distinctive descriptive terms associated with botrytized wines are peach, apricot, quince, tropical fruit, honey, and caramel. The aging process may add more nuances, eg, chocolate, tobacco, dried fruits, raisins, etc.

Listed in historical order, the oldest type of these wines is Tokaji Aszú in Hungary,<sup>1,2</sup> followed by Trockenbeerenauslese (TBA) and Beerenauslese wines in Germany and

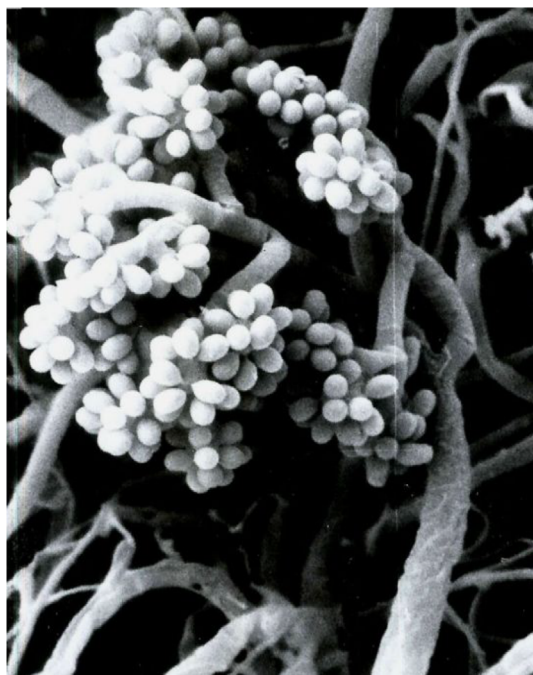
Correspondence: Ildikó Magyar  
Department of Oenology, Institute of  
Viticulture and Oenology, Szent István  
University, 45 Ménesi út, Budapest  
H-1118, Hungary  
Tel +36 1305 7347  
Email Magyar.Ildiko@kertk.szie.hu

Austria.<sup>3,4</sup> Other famous names are Sauternes<sup>4</sup> and “Sélection de Grains Nobles” wines in France. There is an increasing interest in producing botrytized sweet wines in Australia and South Africa, while they are also produced occasionally in California.<sup>1</sup> Recently, the benefits of botrytization have become well recognized and utilized in making various passito wines in Italy (Picolit and Recioto), even in dry styles (Amarone).

Since the 1950s, extended research has been carried out on botrytized wine specialties. The technology, biochemistry, and special characteristics of botrytized sweet wines have recently been reviewed by several authors.<sup>1,3,4</sup> While briefly summarizing the most important and previously confirmed knowledge in this field, the aim of this review is to give an overview of the most recent research on botrytized wines. In accordance with the topics of current publications, the focus of this review is on the interaction of the grape and *B. cinerea* during noble rot, the microbiological diversity of botrytized wines, and the sensory and chemical aspects of their aroma composition.

## The botrytization process

*B. cinerea* (Figure 1) generally is very detrimental to wine quality when it causes bunch rot, also known as gray rot/mold, causing significant economic loss. Under particular conditions, *B. cinerea* generates a special form of infection called noble rot, which highly improves the quality of the grape.



**Figure 1** Scanning electron micrographs of *Botrytis cinerea* conidiophore developing on the surface of noble rotted berry.

The main factors that lead to noble rot are 1) appropriate climatic and microclimatic conditions, 2) appropriate grape variety (ripening time, anatomy of berry and bunch, phytoalexin production, etc), and 3) full or over-ripeness of the grape at the time of *Botrytis* invasion (when the vascular connection between the vine and berry ceases). There are only a few geographical–viticultural environments where all these conditions coincide relatively frequently.

The noble rot can be summarized as an interaction and balance between the enzymatic activity of *B. cinerea* and the concentrating effect of physical dehydration on the grape berry. Several authors have described the infection process, which has recently been reviewed,<sup>1,5</sup> and hence only a brief summary is given in this review. Since *Botrytis* has limited ability to digest the intact cuticle of the berry skin, penetration occurs through stomata, microfissures, or wounds. The growth of the fungus is restricted to the outer layers of the epidermis, digesting the skin, extracting chemical compounds from the berry, and introducing metabolic products into the juice. The permeable skin supports the evaporation of water during sunny and windy days, which results in shriveling of the berry and several-fold concentration of the juice. The sugar content of the botrytized grape commonly reaches 350 g/L in Sauternes<sup>6</sup> or even ~700 g/L,<sup>1,2</sup> if the shriveling process is intensive, as in Tokaj (Figure 2).

*Botrytis* produces numerous hydrolase and oxidase enzymes, which transform many components of the grape tissue and the juice. Oxidation of glucose leads to production of glycerol, >7 g/L,<sup>6</sup> but it may exceed 30 g/L after further berry dehydration, eg, in Tokaji Aszú berries and TBA grapes.<sup>1,3</sup> Other sugar alcohols (arabitol, mannitol, sorbitol, and inositol) and various carbonyl compounds are



**Figure 2** Selective picking of botrytized and shriveled (“aszú”) berries in Tokaj.  
**Note:** Photo courtesy of Dr Zsuzsanna Bene.

also produced. Part of the glucose is oxidized to gluconic acid, considered as another *Botrytis* indicator; however, its elevated level (up to 2–3 g/L) in botrytized berry can be partially explained by the activity of acetic acid bacteria. Taking part in this reaction is a glucose oxidase (GOX), not present in healthy grapes, but typical of botrytized grapes.<sup>7</sup> Via oxidation of glucose to gluconic acid, it releases H<sub>2</sub>O<sub>2</sub>, which can generate further oxidation reactions, such as oxidation of tartaric acid to glyoxylic acid, ethanol to acetaldehyde, and glycerol to glycerol aldehyde. These compounds will further react with catechins and proanthocyanidins, influencing the color and thus the visual quality of the wine. GOX remains stable in wine conditions as it is relatively resistant to sulfites, and ascorbic acid and bentonite have little effect on its activity.<sup>7</sup>

One of the most studied enzyme families of *Botrytis* is an aggressive polyphenol oxidase (PPO) enzyme group called laccase.<sup>6–8</sup> While GOX has an indirect effect on polyphenols, the direct role of PPO in the oxidation of a wide scale of phenols has been known for a long time.<sup>8</sup> Although PPO is more stable in wine than the polyphenol oxidase enzymes of the grape, it is more sensitive to SO<sub>2</sub> than GOX.<sup>7</sup>

*Botrytis* significantly influences the composition of nitrogenous substances in grapes. While the yeast-assimilable nitrogen content decreases and the amino acid pattern changes,<sup>3</sup> production of exocellular fungal enzymes increases the protein content of the juice.<sup>6,9</sup> A further specialty is a particular polysaccharide composition.

More recent studies focus not only on the enzymatic activities of *Botrytis* in the grape but also on the metabolic response of the plant to *Botrytis* attack.<sup>9,10</sup> Changes in the polyphenol composition of the berry result from a combination of the fungal PPO and GOX activity and the metabolic response of the grape to fungal infection. The total phenol content either decreases<sup>11,12</sup> or possibly increases in highly dehydrated berries due to concentration. Flavonoids are the most important group of phenolics in wine. A significant accumulation of several flavonoid glycosides and flavanones was detected in noble rotted berries of Sémillon grape but the production of flavones and flavonols was suppressed during noble rot.<sup>10</sup> In contrast, the presence of the fungus on Chenin blanc grape seems to induce, from one side, an increase in flavonols, such as catechin, epicatechin, and epicatechin gallate, and from the other side, a decrease in caftaric acid, coutaric acid, quercetin-3 glucoside, and kaempferol-3 glucoside.<sup>12</sup> From the results, it seems that changes in the flavonoid composition show varietal influences and need further research.

The plant responds to the fungal infection by producing more stilbenes such as resveratrol and its derivatives, which act as phytoalexins against the fungus. The evolution of these compounds depends on the grape variety and on the development of noble rot.<sup>10</sup> In the last stage of noble rot, viniferin and astilbin could be used by the plant against *Botrytis*. However, the final level of stilbene derivatives (*trans*-astringin, *trans*-resveratrol, *trans*-piceid, etc) is generally low in the botrytized juice and wine, probably because *Botrytis* enzymes of the laccase group, such as stilbene oxidase, are able to transform these molecules to oxidized compounds, which was more extensively reviewed by Teissedre and Donèche.<sup>4</sup>

In a significant, comprehensive study, Blanco-Ulate et al.<sup>10</sup> have found desirable interaction between a fruit and a fungus that stimulates pathways otherwise inactive in white-skinned berries. Using transcript and metabolite analysis of the grape tissue, they identified several genes that were upregulated due to *Botrytis* infection. They showed that noble rot stimulated the synthesis of anthocyanins in white-skinned grape (Sémillon), contributing to the pink and bluish color of the affected berries. Biosynthesis of stilbenes, terpeneols, and fatty acid aroma precursors was also stimulated by *Botrytis*.

Modification of grape metabolism by *Botrytis* concerns the aroma potential of the grape as well, which was extensively studied by Thibon et al.<sup>13</sup> Certain grape varieties (Sauvignon blanc, Sémillon) contain cysteine-S conjugate molecules, which are precursors of several important thiols released by yeasts during fermentation.<sup>13</sup> These cysteinylated precursor compounds are *S*-3-(hexan-1-ol)-L-cysteine (P-3SH), *S*-3-(pentan-1-ol)-L-cysteine (P-3SP), *S*-3-(heptan-1-ol)-L-cysteine (P-3SHp), and *S*-3-(2-methylbutan-1-ol)-L-cysteine (P-2M3SB) and are converted by yeasts to strongly odiferous thiols in the wine (refer the “Aroma composition of botrytized wines” section).<sup>13,14</sup> These precursors are present in the healthy grape as well, but their quantities dramatically increase due to noble rot. The botrytization process increased the P-3SH level in grapes ~100-fold in 1 week, compared to healthy grapes.<sup>15</sup> The production pathway of this molecule was elucidated by Thibon et al.<sup>16</sup> In model experiments using cell cultures, it was demonstrated that grapevine cells were able to produce P-3SH and that the presence of *B. cinerea* considerably increased the precursor level (up to 1,000-fold).

Since the noble rotted grape is superior to the withered grape in terms of wine quality, discriminating them objectively would be valuable from a practical standpoint. The main aim of several studies is to identify possible markers of *Botrytis* infection in dehydrated grapes.

PPO activity is generally accepted as an index of noble rot infection,<sup>6</sup> although some controversial results have been presented.<sup>17</sup> Using monoclonal antibodies, highly sensitive and specific immunoassays have also been elaborated, which are rapid and user friendly, supporting practical application in the wineries.<sup>18</sup> More recently, protein determination by mass spectrometry analysis allowed researchers to identify specific proteins of *B. cinerea* that represent potential markers of the presence of the fungus in the withered grapes.<sup>19</sup>

Additionally, physical properties might be appropriate for differentiating botrytized and withered grapes. Instrumental measurements of chromatic parameters (CIEL\*a\*b\*) on the berries of white grapes proved that only blue dehydrated grapes showed a chemical and volatile composition typical of the noble rot infection. It is of great relevance for wineries where passito wine production is carried out on a small scale, and manual selection of green, orange, and blue berries from the stalk enables winemakers to differentiate the wine characteristics.<sup>20</sup> Measuring various physical parameters (eg, mechanical resistance of the skin, color, etc) the combination of puncture and compression tests with color (CIEL\*a\*b\* parameters) showed a clear differentiation of botrytized berries in Chenin blanc grape (Loire Valley) and appeared to be the most adequate to establish markers of noble rot evolution.<sup>12</sup> Phenolic composition was another discriminating marker in this study. Recently, the applicability of electronic tongue is under study for differentiation of botrytized and healthy juice or wine.<sup>21</sup>

## Induced botrytization

The rare occurrence and uncertainty of noble rot have for a long time motivated researchers to obtain induced and/or controlled botrytization. Moreover, global climatic change will severely impact the delicate equilibrium needed for natural noble rot, a point which has to be considered in future research.<sup>5</sup>

Several promising experiments were reported on artificial postharvest infection of grape or juice. These methods had not been in practice, probably due to the high costs at that time.<sup>5</sup> However, vineyard infection has been applied with success in the Napa valley, California, where a natural *Botrytis* isolate from Sémillon grape was used as a source of inoculum to induce noble rot in the production of Dolce Wine (Oakville, CA, USA). This strain was successfully used for artificial infection in the vineyard in several subsequent years, and the noble rotted grape has been used for commercial wine production as well as for several interesting research studies on botrytization.<sup>10,22–24</sup> The draft genome sequence of this strain (BcDW1) has recently been published.<sup>10</sup>

Induction of *Botrytis* infection during postharvest withering of the grape has been increasing as a strategy in making straw (passito) wines (eg, Amarone and Recioto) in Italy. Although these wines are traditionally made of fully ripened but nonbotrytized grapes that are dehydrated in drying chambers after harvest, *Botrytis* infection occasionally may occur either in the vineyard or during withering. The incidence of spontaneous *Botrytis* infection is extremely uncertain, depending on seasonal conditions and withering techniques.<sup>10,11,25,26</sup> Both research and practical investigations on different passito wine types have proved that *Botrytis* infection of the withering grape significantly improves aroma composition. Artificial postharvest infection by selected local *B. cinerea* strains during grape withering has been demonstrated to be suitable by several authors, and implementing this procedure in drying fruit rooms to standardize the level of grape botrytization is encouraged.<sup>11,27,28</sup> For the controlled postharvest botrytization, several methods have been developed. In an internationally patented automatic system, the grape treatment is carried out in a drying room in various drying cycles. The first cycle involves dehydration at 76% relative humidity (RH) and 11°C for 30 days, followed by exposure to 100% RH for 7 days and a final cycle of 76% at 8–10°C.<sup>29</sup> This method successfully induces activity of *B. cinerea* latently present on the bunches, and the resulting wine has characteristics typical of botrytized wines.<sup>5</sup> Another method involves artificial infection of the grape with a commercial *Botrytis* inoculum in an induced mist and decreasing RH to 50% after 3–4 days. The whole botrytization and withering process requires 15–25 days, depending on the grape variety. Besides improving sensory characteristics of the passito wines, postharvest botrytization technologies offer a way to produce botrytized wines in the regions where it is not naturally possible or in traditional noble rot areas during bad seasons.<sup>5</sup>

## Diversity of yeast and bacterium biota of botrytized grapes and wines

The microbiology of botrytized winemaking, with special regard to the alcoholic fermentation, is more complex than that of normal vinification. Its special features involve the modified composition of yeasts and bacteria on the botrytized grape and the high sugar content as well as other difficult environmental conditions of botrytized must and presence of possible inhibitors. As a result, atypical microbial communities of botrytized wines can be expected and are actually found.



Studies on the yeast biota of botrytized grapes in various countries have revealed a strong presence of *Starmerella bacillaris* (syn *Candida zemplinina*). This is known from very early research, but in various publications the name of the wine strains of the same species has been changed several times from the original *Torulopsis bacillaris*<sup>30</sup> to *C. stellata*<sup>31</sup> and then to *C. zemplinina*<sup>32–34</sup> due to the changes in yeast taxonomy. Recently, a new classification has been suggested reinstating the species *S. bacillaris*, with the previous name *C. zemplinina* as an obligate synonym.<sup>35</sup> In this review, however, we continue to use the synonymous name, since most of the enological studies refer to *C. zemplinina*.

*C. zemplinina* population significantly increases on botrytized berries in Tokaj during postharvest storage<sup>33</sup> and likewise grows during withering of grapes for passito wine production;<sup>36</sup> hence it has an important role in fermentation. *C. zemplinina* has several interesting enological properties: sugar tolerance,<sup>32,34</sup> fructophilic character,<sup>22,37–39</sup> good glycerol production,<sup>36,37,39,40</sup> moderate or low acetic acid production,<sup>39</sup> and a particular aroma profile.<sup>40,41</sup> These properties make *C. zemplinina* a good candidate for starter culture in combination with *Saccharomyces* strains – not only in botrytized wines but also in normal wines as well.<sup>40–44</sup>

In botrytized wine fermentation, *C. zemplinina* frequently initiates the spontaneous fermentation and survives long after *Saccharomyces* strains have begun to dominate, particularly at lower temperatures.<sup>22,45</sup> Its main contribution to the quality of botrytized wine is to lend a beneficial modification of the glucose–fructose ratio and enhance glycerol production.<sup>38</sup> Its importance in the aroma composition of Tokaji Aszú was not confirmed,<sup>46</sup> but seems significant in other wine styles.<sup>40</sup>

Various molecular genetic methods provide more and more possibilities for following the population dynamics of yeasts during fermentation. Using denaturing gradient gel electrophoresis (DGGE) during Dolce wine fermentation in California, *C. zemplinina* was detected as the dominant yeast throughout the early fermentation in certain vintage years.<sup>22</sup> This species persisted throughout, with *S. cerevisiae* starting to appear only later in the fermentation. In other years, using terminal restriction fragment length polymorphism (RFLP) in the same wine type, the *C. zemplinina* population was not as pronounced at the beginning, although it was found to increase in the microbiota by the end of fermentation.<sup>23</sup> Long prevalence of *C. zemplinina* was also found in fermenting Tokaji Aszú,<sup>38</sup> Greek botrytized wine,<sup>45</sup> and passito wine.<sup>36</sup>

Although *C. zemplinina* is known to be particularly associated with high sugar musts or wines, it is common on nonbotrytized grapes as well, although in lower populations.

Recent genotyping of 163 wine-related strains from seven countries did not show any relationship between the genetic diversity and sweet/dry wine origin, suggesting that high sugar concentration in winemaking has no impact on *C. zemplinina* selection and adaptation. These surprising results indicate that *C. zemplinina* is not under selective pressure in winemaking environment.<sup>47</sup> The reasons for its prevalence in botrytized wine fermentations need further explanation.

On the botrytized grape, other sugar-tolerant, non-*Saccharomyces* species, including *Zygosaccharomyces bailii*, *Hanseniaspora species*, *Metschnikowia pulcherrima*, and *Candida lactis-condensi*,<sup>23,33</sup> *Issatchenkia spp.* or *Kluyveromyces dobzhanskii*,<sup>45</sup> are present and contribute to the fermentation, although to a lesser extent and for a shorter time than *C. zemplinina* in general. Several *M. pulcherrima* are capable of making a positive contribution to volatile thiol release (3SH) in wines, essentially during the prefermentation stage in winemaking, when this microbiological subpopulation is dominant.<sup>48</sup> Apart from *C. zemplinina*, a persistent dominance of *Z. bailii* throughout the fermentation was also reported in *Botrytis*-affected fermentation.<sup>45</sup> Being sugar tolerant and resistant to various chemical preservatives, this species is considered an important spoilage yeast in winemaking.

However, the major part of fermentation of botrytized wines is normally dominated by *Saccharomyces* species, just as in normal wines. Concerning the population dynamics in the course of spontaneous fermentation, a late appearance and getting dominance of *Saccharomyces* strains are typical.<sup>23,38</sup> The *Saccharomyces* biota of botrytized wines seems different from that of normal wines. The most striking characteristic is the high frequency of *S. bayanus* var. *uvarum* strains in botrytized musts and fermentations.<sup>49</sup> While *S. bayanus* var. *uvarum* is a relatively rare wine yeast in most wine regions, its significant presence was confirmed in Tokaj,<sup>31,38,50</sup> Sauternes,<sup>51</sup> and Amarone<sup>52,53</sup> wines as well. Whether these *Saccharomyces* strains are generally present on the botrytized grape is not completely clear. From the reports, it seems that these strains can be isolated from the grape only after selective enrichment, if at all.<sup>23,33</sup> Various studies, using different molecular methods, report on a huge genetic diversity of *Saccharomyces cerevisiae* in botrytized wines either in Tokaj<sup>31,50</sup> or in Sauternes<sup>51,54</sup> or in north Italy.<sup>52</sup> In contrast to this, *S. bayanus* var. *uvarum* exhibits low polymorphism in chromosome size<sup>49</sup> and also its mtDNA was found to be less polymorphic than that of *S. cerevisiae*.<sup>49</sup> Enological properties of *S. bayanus* are significantly different from those of *S. cerevisiae*,<sup>37,53,55</sup> especially regarding its high glycerol and succinic acid production and particular aroma profile (with

high 2-phenylethanol production), as well as a pronounced cryophilic character. Viniculture and winemaking at low temperatures seem to be the specific ecological niche for *S. bayanus* var. *uvarum*.<sup>49</sup>

To accelerate and control the difficult fermentation process, yeast starter cultures have long been used in fermentation of Sauternes and BA/TBA, wines, as well as in the newer style botrytized wines. This practice is spreading in Tokaji Aszú fermentation too. The predictability can be improved and the microbiological diversity of fermentation can be reduced in this way, and the impact of the local yeast biota still remains significant.<sup>23,38</sup> Of the commercial starter cultures, only a few strains can cope with the difficult conditions of botrytized must, so finding the appropriate strain requires thorough consideration and practical experiments. Selection of local strains, which are well adapted to the particular conditions, is an important perspective.<sup>38,56</sup> The use of *S. bayanus* strains as single starter cultures seems sufficient only at low temperature, because the local *S. cerevisiae* strains easily outgrow them. Combined applications of *S. bayanus* and *S. cerevisiae* and/or non-*Saccharomyces* species are under study.<sup>57</sup>

Selecting a local strain or choosing commercial yeast appropriate for botrytized wine fermentation has special criteria, in addition to high sugar tolerance. Since the high sugar content and *Botrytis* glucans dramatically enhance the acetic acid production of yeasts, a low acetic acid production is essential.<sup>1,38</sup> The diversity of different *Saccharomyces* strains in enological traits involves aroma production (higher alcohols, fermentation esters, etc), including their capacity to release volatile thiols by cleavage of S–C bonds in the precursor molecules present in grapes.<sup>14</sup> This capacity is particularly important in fermentation of botrytized Sauvignon blanc and Sémillon wines.

In contrast to yeasts, the bacterium biota of botrytized wines is not well studied. It has long been known that acetic acid bacterium population reaches high levels on the digested berry skin, leading to an elevated acetic acid, keto-gluconic, and gluconic acid concentration in the juice, but little is known about other taxa. A recent study utilizing a next-generation sequencing technique<sup>24</sup> has determined the bacterial diversity of botrytized wines to be far higher than previously realized. Several previously unreported bacteria (eg, *Chryseobacterium*, *Methylobacterium*, *Sphingomonas*, etc) were identified on wines in 2 years of Dolce fermentation. As expected, *Saccharomyces* inoculation exerted selective pressure on bacterial diversity in these fermentations, most notably suppressing abundance of acetic acid bacteria

(*Gluconobacter*). In both the vintages studied, Rhodospirillales (predominantly *Acetobacter*, *Gluconobacter*, and *Gluconacetobacter*) were the most dominant bacteria detected, with secondary populations of Lactobacillales. The LAB community of Dolce was also abnormal compared to normal wines, comprising primarily *Leuconostoc* and *Lactococcus* in addition to the more typical *Lactobacillus* and *Pediococcus*; notably, *Oenococcus* was entirely absent.

## Aroma composition of botrytized wines

The chemical changes induced by noble rot and complemented and combined with the yeast activities during alcoholic fermentation result in a special, very complex chemical composition of botrytized wines. The main characteristics have been well studied and reviewed by several authors.<sup>1,3,6</sup> The most important differences compared to normal wines involve residual sugar content from the grape, high glycerol and gluconic acid concentrations (considered *Botrytis* indicators in the juice and wine), modified organic acid pattern, high level of carbonyl compounds (with high sulfite binding capacity), and low level of yeast-assimilable nitrogen. A further specialty of botrytized wines is the higher polyphenol content and antioxidant capacity as well as a particular polysaccharide and protein composition arising from the noble rotted grape. These properties lead to technological difficulties in stabilization and clarification, as reviewed in earlier publications.

Concerning the studies on the chemical composition of botrytized wines, the most significant development can be found in the aroma research. The particular aroma ranges of various botrytized wines are determined by several interacting factors. The primary factors are the *Botrytis* activity and the respective metabolic responses of the grape. In addition, alcoholic fermentation (yeast, temperature, and nutrients) and maturation of wines (time, level of oxidation, sulfite addition, and type of cooperage) significantly influence the final style and sensory character of these wines.

The volatile compounds identified in sweet botrytized wines belong to numerous chemical classes (Table 1). Earlier research showed that while the terpene content typically decreases in these wines, numerous hydroxy-, oxo-, and dicarboxylic acid esters, acetals, and lactones form, which are present in lower concentrations or absent in normal wines.<sup>58–61</sup>

The first research on the chemical nature of botrytized aroma character focused mainly on heterocyclic odoriferous compounds. One of the first compounds identified as a key odorant in these wines was sotolon (4,5-dimethyl-3-hydroxy-2(5H)-furanone), having a sweetish, caramel, or

**Table 1** Volatile compounds significantly associated with botrytized wines as characteristic aroma substances

Compound	Sensory descriptors	Wine types and references
<b>Aldehydes</b>		
Benzaldehyde	Bitter almond	Recioto di Soave <sup>27</sup>
Phenylacetaldehyde	Honey	Sauternes, Amarone, and Recioto <sup>26,64,66</sup>
Furfural	Almonds	Recioto di Soave <sup>27,28</sup>
Methional	Baked potatoes	Sauternes <sup>64</sup>
<b>Other carbonyl compounds</b>		
Beta-damascenone	Fruity, quince, and canned apple	Sauternes and sweet Fiano <sup>64,68,71</sup>
Homofuraneol	Caramel, cotton candy, and sweet	Sauternes and Recioto di Soave <sup>64,68</sup>
Furaneol	Caramel	Sauternes <sup>64,68</sup>
Norfuraneol	Caramel	Sauternes and Amarone <sup>64,66</sup>
<b>Alcohols</b>		
2-Phenyletanol	Rose and floral	Sauternes <sup>64,71</sup>
3-Mercaptohexan-1-ol	Grapefruit	Sauternes <sup>64,69</sup>
Benzyl alcohol	Berry, cherry, and walnut	Sweet Fiano and Recioto <sup>28,71</sup>
1-Octen-3-ol	Mushroom	Sweet Fiano, Amarone, and Recioto di Soave <sup>27,28,66,71</sup>
Methional	Potatoes and soy	Amarone <sup>66</sup>
<b>Esters</b>		
Ethylhexanoate	Pineapple, green apple, and banana	Sauternes <sup>64,68</sup>
2-Phenylethyl acetate	Fruity, flowery, and honey	Amarone <sup>66</sup>
<b>Lactones</b>		
Gamma-nonolactone	Peach and apricot	Sauternes, sweet Fiano, Tokaji Aszú, and Recioto <sup>27,59,60,64,68,71</sup>
Delta-decalactone	Coconut	Tokaji Aszú, sweet Fiano wine, and Sauternes <sup>58–60,64,71</sup>
Gamma-decalactone	Peach and apricot	Tokaji Aszú, sweet Fiano, and Sauternes <sup>58–60,64,68,71</sup>
Sotolon (3-hydroxy-4,5-dimethylfuran-2(5H)-on)	Caramel, curry, and nut	Sauternes <sup>62,64,70</sup>
Sherry lactone (5-hydroxy-4-hexanolide)-isomer 2		Amarone <sup>66</sup>
2-Nonen-4-olide	Mint and overripe orange	Sauternes <sup>67</sup>
<b>Volatile thiols</b>		
3-Sulfanilhexan-1-ol	Fruity, rhubarb, and grapefruit	Sauternes <sup>68–70</sup>
3-Sulfanylpentan-1-ol	Grapefruit	Sauternes <sup>70</sup>
3-Sulfanylheptan-1-ol	Citrus	Sauternes <sup>70</sup>
2-Methyl-3-sulfanylbutan-1-ol	Raw onion	Sauternes <sup>27,70</sup>
<b>Terpenes</b>		
Nerol, geraniol, and linalool	Orange flower	Sweet Fiano <sup>71</sup>
Alpha-terpineol		Sauternes <sup>68</sup>
4-Terpineol	Floral and lilac	Amarone <sup>66</sup>
<b>Others</b>		
Vitispirane	Camphor	Sweet Fiano <sup>71</sup>
N-(3-Methylbutyl)-acetamide	Vinegar	Amarone and Recioto <sup>27,28,66</sup>

nut taste.<sup>62</sup> Its presence has been repeatedly confirmed in botrytized wines.<sup>63,64</sup> However, it is also a typical compound in some nonbotrytized wines such as flor sherry and fortified sweet wines such as Port.<sup>65</sup>

Among heterocyclic compounds, beta-lactones (coco-nut) and gamma-lactones (peach and apricot)<sup>20,59,61,64,66</sup> were confirmed as key aroma compounds. The use of the gas chromatography/mass spectrometry/olfactometry technique has significantly contributed to the understanding of botrytized aroma character. More recently, a new lactone, 2-nonen-4-olide, was identified in Sauternes wines.<sup>67</sup> While this molecule alone presents minty and fruity nuances, together with other volatile compounds it contributes to the

overripe fruit (particularly orange) aroma and typicality in Sauternes sweet wines.

In addition to lactones, higher alcohols, aldehydes, and esters, eg, ethyl-hexanoate (pineapple and banana), phenylethanol (rose), phenylacetaldehyde (honey), beta-damascenone (fruity, quince, and canned apple) and different furanones (caramel), have important roles in the distinct aroma of botrytized wines.<sup>64,68</sup> These compounds, however, do not fully explain the citrus-like nuances. In the last decade, studies on Sauternes wines using gas chromatography–olfactometry methods revealed the importance of polyfunctional thiols in this respect, especially sulfanylaldehydes, sulfanyl-alcohols, and sulfanylesters.

Volatile thiols are extremely strong odorants; many of them bad smelling (eg, onion), but a few have pleasant, citrus-like odor. Several volatile thiols are an important part of the varietal character of Sauvignon blanc and Semillon wines made from healthy grapes as well.<sup>69</sup> Tominaga et al<sup>69</sup> presented first that 3-sulfanylhexas-1-ol (3SH) is present at very high concentration and contributes to the citrus/grapefruit nuance of Sauternes sweet wines. During the last decade, several new volatile thiols have been identified as important odorants of Sauternes wines, such as 3-methyl-3-sulfanylbutanal (bacon-petroleum),<sup>63</sup> 3-sulfanylpentan-1-ol (3SP, citrus and sulfur), 3-sulfanylheptan-1-ol (3SHp, grapefruit), and 2-methyl-3-sulfanylbutan-1-ol (2M3SB, raw onion).<sup>70</sup> Thiols are practically not present in grape, only in wines, and are released from precursor molecules during alcoholic fermentation, as presented in a previous section. *Botrytis* infection highly enhances the formation of the precursors in the grape, and the various yeast strains have a further impact on the final composition of thiols in the wine.

These important findings on the role of volatile thiols in *Botrytis* aroma originated from the studies of botrytized Sauvignon blanc and Semillon wines in the Sauternes region. Whether these molecules have similar importance in botrytized wines from other grape varieties (eg, Furmint in Tokaj or Riesling, Gewürztraminer in Germany, Chenin blanc in South Africa) has remained unclear and needs further research.

As discussed in the “Induced botrytization” section, there is an increasing interest in improving the aroma composition with notes of botrytization in some passito wines. This might occur naturally on the grapevine or as a postharvest process during the withering of the grape in climatized chambers, but artificial botrytization is also a strategy in some wineries. Table 2 shows the comparison of the main volatile compounds most affected by *Botrytis* in a dry red passito wine Amarone, when healthy grape or partially botrytized grape was vinified.<sup>66</sup> These data clearly show the great impact of *Botrytis* and with respect to the various compounds, they generally confirm the earlier findings obtained from traditional sweet botrytized wines. In another study, the combined effect of *Botrytis* and the yeast strain was studied in Recioto di Soave microvinifications.<sup>27</sup> Although the effect of the botrytization on wine aroma was greater (revealing *Botrytis* products such as 1-octen-3-ol, gamma-nonolactone, and benzaldehyde) than that of yeasts, the contribution of the latter also proved significant. Large variations between yeasts (local strains or commercial starters) were observed for several compounds (eg, ethyl esters, lactones, phenylacetaldehyde, isovaleric acid, and *N*-(3-methylbutyl)acetamide). The results indicate the importance of the choice of strain to modulate wine flavor in passito wines.

**Table 2** Aroma compounds detected by SPE-GC-MS in dry, red Amarone wines obtained from healthy grape must (W-100) and from mixed healthy and botrytized grape musts at different percentages (W-80: 20% botrytized, and W-60: 40% botrytized)

Aroma compound	W-100	W-80	W-60
<i>trans</i> -3-Hexenol	(23±2) <sup>a</sup>	(28.0±0.9) <sup>b</sup>	(30±2) <sup>b</sup>
Methionol	(176±15) <sup>a</sup>	(483±116) <sup>b</sup>	(345±87) <sup>ab</sup>
1-Octen-3-ol	(9±3) <sup>a</sup>	(15±1) <sup>b</sup>	(16±2) <sup>b</sup>
Isoamyl acetate	(16±3) <sup>a</sup>	(30±5) <sup>b</sup>	(36±2) <sup>b</sup>
2-Phenylethyl acetate	(32±2) <sup>a</sup>	(42±4) <sup>b</sup>	(50±5) <sup>b</sup>
Ethyl 2-hydroxyisovalerate	(23±3) <sup>a</sup>	(35±2) <sup>b</sup>	(40±2) <sup>b</sup>
Ethyl 2-hydroxy-4-methylpentanoate	(115±4) <sup>a</sup>	(161±11) <sup>b</sup>	(179±11) <sup>b</sup>
Ethyl phenylacetate	(5.2±0.6) <sup>a</sup>	(12±2) <sup>b</sup>	(20±2) <sup>c</sup>
Benzaldehyde	(12±3) <sup>a</sup>	(17±4) <sup>a</sup>	(21±4) <sup>b</sup>
Phenylacetaldehyde	(0.2±0.1) <sup>a</sup>	(2.9±0.7) <sup>b</sup>	(2.8±0.6) <sup>b</sup>
Norfuraneol	(56±6) <sup>a</sup>	(79±5) <sup>b</sup>	(81±12) <sup>ab</sup>
<i>trans</i> -Furanic linalool oxide	(5.0±0.3) <sup>a</sup>	(6.4±0.7) <sup>ab</sup>	(7.2±0.4) <sup>b</sup>
<i>cis</i> -Furanic linalool oxide	(4.1±0.4) <sup>a</sup>	(5.5±0.4) <sup>b</sup>	(6±1) <sup>ab</sup>
4-Terpeneol	(10±2) <sup>a</sup>	(13±1) <sup>a</sup>	(18±2) <sup>b</sup>
Sherry lactone (isomer 2)	(215±10) <sup>a</sup>	(311±33) <sup>b</sup>	(386±32) <sup>c</sup>
Methyl vanillate	(10±1) <sup>a</sup>	(13±1) <sup>b</sup>	(13.9±0.3) <sup>b</sup>
Ethyl vanillate	(78±6) <sup>a</sup>	(112±25) <sup>ab</sup>	(127±12) <sup>b</sup>
Homovanillic acid	(11.3±0.5) <sup>a</sup>	(14±1) <sup>b</sup>	(15.7±0.6) <sup>b</sup>
<i>N</i> -(3-Methylbutyl)-acetamide	(65±3) <sup>a</sup>	(915±142) <sup>b</sup>	(1,878±138) <sup>c</sup>
Not identified	(1,995±179) <sup>a</sup>	(2,598±369) <sup>ab</sup>	(3,287±80) <sup>b</sup>

**Notes:** Of the 53 compounds determined only the compounds whose concentrations varied statistically >30% in botrytized wines with respect to healthy grape wine are shown. Adapted with permission from Fedrizzi B, Tosi E, Simonato B, et al. Changes in wine aroma composition according to botrytized berry percentage: a preliminary study on Amarone wine. *Food Technol Biotechnol*. 2011;49(4):529–535.<sup>66</sup> Mean values with different letters in superscript within a row are significantly different ( $P<0.05$ ).

**Abbreviation:** SPE-GC-MS, gas chromatography-mass spectrometry.

Botrytized wines are typically aged for a long time either in barrels (like Tokaji Aszú) or in bottles (Sauternes, TBA wines). The length, the oxygen ingress, the level of SO<sub>2</sub>, and the material of the cooperage have a huge impact on the aroma composition of the new botrytized wines. Although the variations in these factors significantly determine the style of the finished wine, these effects are not as fully researched as the impact of botrytization.

In a survey on aged Sauternes wines,<sup>68</sup> most polyfunctional thiols (3-sulfanylpentyl acetate, 2-sulfanylethyl acetate, 3-methyl-3-sulfanylbutanal, etc) completely disappeared after 2 years of bottle aging in a cellar. Only 3-sulfanylhexas-1-ol (3SH) was found in aged samples at concentrations above its threshold value. In contrast to the thiols, most other key odorants found in the young noble rot wine were still detected 5–6 years after harvest. In the same study, a new lactone, abhexon, was found, for the first time, to be synthesized during bottle aging.

Fedrizzi et al<sup>25</sup> compared Amarone wines from botrytized and nonbotrytized grapes, under oxidative and



anaerobic conditions. This research highlighted peculiar evolutions for several volatile compounds in botrytized wine. In particular, benzaldehyde showed a tangible increment during the oxidative aging, with a rate much higher than that reported for non-*Botrytis* red wines. On the other hand, several sulfides disappeared after just 15 days of oxidative aging.

## Conclusion

Noble rot of the grape is a complex microbiological and biochemical process, which has long been under investigation. The older studies elucidated the direct role of *Botrytis* enzymes in transformation of the substrates present in grape. While confirming most of the previous findings, recent publications apply a transcriptomic and metabolomic approach to understand the response of the grape plant to the stress caused by *Botrytis* under the conditions of noble rot. These responses led to a modulation of the grape metabolism (eg, enhanced production of certain polyphenols such as stilbenes and anthocyanins, terpenes, or cysteinylated thiol precursors), which contribute to the distinct composition and sensory character of these wines.

In recent years, benefits of the botrytization process have been thoroughly studied and successfully implemented in the technology of passito wines, where the postharvest withering of grapes in fruit-drying rooms is the key process for the production. Another significant area of current research is the diversity of microbiota and its importance in alcoholic fermentation. Use of carefully selected *Saccharomyces* and non-*Saccharomyces* yeast species as oligo starter culture is a promising direction in the control of the difficult fermentation process.

Thanks to the developments of the analytical methods, aroma composition of botrytized wines is under extensive research, elucidating the various effects (grape, *Botrytis*, dehydration, yeasts, etc). These investigations widely confirmed the old results and presented many new findings.

Producing botrytized wines is a demanding task for winemakers. In spite of significant advances, this area needs further research. The multivariate interactions among grape variety, *Botrytis*, yeast species, wine matrix, and temperature effects make this topic very complex and in need of better understanding. Timing and implementation of harvest, processing the special grape, induction and stoppage of alcoholic fermentation, preventing sweet wines from refermentation during the time of aging, and chemical and microbiological stabilization present the major technological challenges and need further research of an applied nature.

## Disclosure

The authors report no conflicts of interest in this work.

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