CHAPTER 6

Botrytized Wines

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Contents

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Abstract

Botrytized wines are natural sweet wines, produced from grapes that are affected by *Botrytis cinerea* under particular conditions. This rare and special form of fungal infection, called noble rot, includes complex enzymatic conversions and concurrent dehydration of the grape berry and results in a highly concentrated final product. The main characteristics of the noble rotted grapes involve high sugar, acid, glycerol and mineral contents, special polysaccharides, and particular aroma composition, which are thoroughly studied. The saprophytic microbiota of the grapes is also affected. Harvest and vinification of the noble rotted grapes are difficult, having special requirements. Microbiology and biochemistry of the alcoholic fermentation in these wines have been recently studied more deeply. Depending on the grape varieties as well as vinification and ageing technologies, botrytized wines show large diversity in style. Most of them are rich in polyphenols, possessing high antioxidant capacity. Biogenic amine and micotoxine contents of these wines are no public health concerns. This chapter presents the microbiological, biochemical, and technological aspects of noble rot and botrytized wines and discusses the recent findings on these fields.

I. INTRODUCTION

Botrytized wines constitute a distinctive category of natural dessert wines. The residual sugar content of these wines derives from the fermentation of grape juice, affected by the fungus *Botrytis cinerea* under particular environmental conditions. This special fungal infection of the grape is called noble rot (in French: *pourriture noble*; in German: *Edelfäule*). In contrast to the common, detrimental infection by *Botrytis*, called gray rot or bunch rot, noble rot increases grape quality and makes it possible to produce extremely concentrated, aromatic, sweet wines. Fortification is not permitted. Thus, their alcohol content is typically low to medium, arising only from the fermentation of the original sugar content of the juice.

The main difference between botrytized wines and other nonfortified sweet wines, for example, late-harvest wines, icewines (*eiswein*), or straw wines, is the extreme range and richness of the aroma compounds produced by *Botrytis*. Marked differences also exist in some other components (e.g., glycerol, acid composition), due to the microbial activity. According to the descriptors most often applied to these wines, they are characterized by peach, apricot, pear, quince, raisin, and honey flavors, combined with distinctive "botrytis" or *roti* aspects. Another typical feature of botrytized wines is their high acid contents. These prevent them from appearing cloying, even if the sugar content is commonly over 200 g/l.

Beside these basic characteristics, the various types of botrytized wines may possess marked differences in style, depending on the grape variety, the vinification technology, and the length and method of aging. Key technologic and quality parameters for some traditional botrytized wines are given in Tables 6.1 and 6.2.

Botrytized wines have been made for a very long time in Europe, and also are produced in increasing amounts in Australia, New Zealand and South Africa. Although only a few types of botrytized wines are regularly produced, their occasional production is possible in many regions, depending on the weather conditions.

The aim of this review is to give an insight into the diversity of botrytized wines, the biochemical—physicochemical processes of noble rot, and the vinification process, with special regard to the alcoholic fermentation. The health concerns of botrytized wine consumption are also presented and discussed.

II. THE MAIN TYPES OF BOTRYTIZED WINES

A. Tokaji Aszú

The first known wine which was intentionally made from noble-rotted grapes is Tokaji Aszú. It was initially produced in Hungary at least one century earlier than the similar wines in the Rhine valley, and probably two centuries earlier than in Sauternes (Jonson and Robinson, 2001). Tokaj is the name of a town and also a wine district in Hungary. The official appellation of its wines is listed in the European wine register as Tokaj (E-Bacchus database, 2010), although the traditional, local name Tokaji (meaning ''of Tokaj'') can be officially used as well. This is the name preferred by producers and used on the label of the bottles. However, the old English spelling, Tokay, should be avoided, as this name currently refers to wines other than Tokaji, for example, dry wines of Californian, South African or French origin, or certain sweet styles of Australian wine.

There is historical evidence proving that this type of wine has been made since the sixteenth century in the Tokaj-foothills, Tokaj-Hegyalja (Alkonyi, 2000). The vineyards of the region were some of the first to be classified by a royal prescript in 1773 (Bodnar, 2005). By the eighteenth century Tokaji Aszú had been introduced to the courts of kings all over Europe (Jonson and Robinson, 2001; Kirkland, 1996). The geography, grape cultivation, winemaking practice, trade, and scientific knowledge about Tokaj wines of these times have been documented by Szabó and Török (1867), whose work is available in a new reprint published in 2001. The history of the legal regulations concerning Tokaj wine production is presented by Bodnar (2005).

Today Tokaj-Hegyalja includes 5500 ha (13,600 acres) of vineyards, with soil types varying from predominantly red clay to loess with some types of volcanic debris, a mixture of white rhyolite, pumice, and perlite

TABLE 6.1 Comparison of some technological and analytical parameters of traditional botrytized wine types of Hungary, Germany, and Austria

	Tokaji Szamorodni sweet	Tokaji Aszú 3–6 puttonyos	Tokaji Eszencia	Auslese	Beerenauslese	Trocken-beerenauslese	Ausbruck
References of regulations	1, 2, 3	1, 2, 3	1, 2, 3	1, 2, 4/5	1, 2, 4/5	1, 2, 4/5	1, 2, 5
Country	Hungary	Hungary	Hungary	Germany/Austria	Germany/ Austria	Germany/ Austria	Austria
Grape variety	Furmint, Hárslevelű Muscat lunel Zéta Kövérszőlő	Furmint, Hárslevelű, Muscatlunel, Zéta, Kövérszőlő	Furmint, Hárslevelű, Muscat lunel, Zéta	Riesling, Gewürtztraminer, Pinot gris, Scheurebe, etc.	Riesling, Gewürtztraminer, Pinot gris, Scheurebe, etc.	Riesling, Gewürtztraminer, Pinot gris, Scheurebe, Furmint, etc.	Riesling, Furmint Muscat Chardonnay, etc.
Initial sugar content of juice, minimum (g/l/Oechsle/ KMW)[a]	256 g/l	Juice: 205 g/l, Aszú grape: 45°Brix (543 g/l)	543 g/l	Germany: 85–100 Oe[d]/Austria: 21 KMW (256 g/l)	Germany: 110–125 Oe[d]/Austria: 25 KMW (284 g/l)	Germany:150 Oe/ Austria:30 KMW (340 g/l)	27° KMW (300 g/l)
Initial sugar content of juice, typical (g/l)	250–300	250–350[a]	700–800	250–300	300–350	350–400	300–400
Initial actual alcohol content of juice, typical (%, v/v)	0	9–6[b]	0	0	0	0	0
Final sugar content of wine: minimum (g/l)	10	60–150[c]	450	S.A.[e]/N.S.[f]	S.A.[e]/N.S.[f]	S.A.[e]/N.S.[f]	N.S.[f]
Final sugar content of wine: typical	40–70	70–200[c]	600–700	20–70	60–120	100–250	70–200
Final actual alcohol content of wine, minimum (%, v/v)	9	9	N.S.	Germany: 7.0 Austria: 5.0	Germany: 5.5 Austria: 5.0	Germany: 5.5 Austria: 5.0	5.0

Final actual alcohol content of wine, typical (%, v/v)	11–14	10–13	1–3	8–12	6–11	6–10	10–12
Titratable acidity in wine, typical (g/l)	7–8	8–10	15	5–7	6–9	7–10	7–10
Aging time in oak barrel, minimum (year)	1	2	N.S.[f]	N.S.[f]	N.S.[f]	N.S.[f]	N.S[f]
Aging time in oak barrel, typical (year)	1–2	2–5	0–10	0–1	0–1	0–1	0–1
Use of new barrique	Exceptional	Exceptional	No	Exceptional	Exceptional	No	Optional
Total SO_2 content, maximum (mg/l)	350	400	400	350	400	400	400

Regulations (details are given in the list of references):
1. Commission Regulation (2009a), (EC).
2. Commission Regulation (2009b), (EC).
3. Hungarian Wine Law (2004).
4. German Wine Law (1994).
5. Austrian Wine Law (2009).

[a] Local legal measures for sugar content. 1 Oechsle (°Oe) is about 5°Brix; KMW is Klosterneuburger Mostwage, about the same as °Brix).
[b] Juice obtained from maceration of botrytized berry with nonbotrytized must or wine.
[c] Depending on the ‘‘puttony number.’’
[d] Depending on the wine regions of Germany, regulated by Reference 4.
[e] Specified indirectly as the difference between the total alcohol content (the sum of the actual and the potential alcohol), and the actual alcohol content.
[f] Not specified by law/regulation/standard.

TABLE 6.2 Some technological and analytical parameters of French, Australian, South African, and Califorian botrytized wines

	Sauternes and Barsac	Sélection de Grains Noble, Alsace	Sélection de Grains Noble Coteaux du Layon/Coteaux del'Aubance	Botrytis Semillon	Noble late-harvest wines	Late-harvest wines
References of regulations	1, 2, 3	1,2, 4	1, 2, 5/6	7	8	
Country	France	France	France	Australia	South Africa	California
Grape variety	Semillon, Sauvignon blanc Muscat blanc	Gewürztraminer, Pinot gris/ Riesling, Muscat	Chenin blanc	Semillon	Chenin blanc Sauvignon Semillon	Semillon, Sauvignon
Initial sugar content of juice, minimum (g/l)	221	279/256	294	N.R.	28 Balling	N.R.
Initial sugar content of juice, typical (g/l)	300–350	250–350	350–400	350–450	350–400	300–400
Initial actual alcohol content of juice, typical (%, v/v)	0	0	0	0	0	0
Final sugar content of wine, minimal	N.R.	N.R.	34–68[a]	N.R.	50	N.R.
Final sugar content of wine, typical	50–150	50–150	50–150	170–220	100–180	70–140
Final actual alcohol content of wine, minimal (%, v/v)	12	N.R.	11–12/11	9	N.R.	N.R.
Final actual alcohol content of wine, typical (%, v/v)	13–14	12–13	11, 5–13	10–11.5	10–12	13–14.5
Titratable acidity in wine, typical (g/l)	6–8	7–10	7–9	9–10	5–7	5–7.5
Aging time in oak barrel, minimal (year)	N.R.	N.R.	N.R.	N.R.	N.R.	N.R.

Aging time in oak barrel, typical (year)	1–3	0–1	0–1	1–2	0–1	3–5
Use of new barrique	Typical	Exceptional	Exceptional	Typical	Optional	Optional
Total SO_2 content, maximum (mg/l)	400	400	400	300	N.R.	200

Regulations (details are given in the list of references):
1. Commission Regulation (2009a), EC.
2. Commission Regulation (2009b), EC.
3. Décret (2009), France.
4. Décret (2007), France.
5. INAO (2007), France.
6. INAO (2003), France.
7. Food Standards Australia and New Zealand (2011).
8. South African Wine Law (1989).

[a] Depending on the appellations.

(Alkonyi, 2000). The microclimate of the Tokaj wine district is beneficially influenced by the sheltering effect of the Zemplén hills and by the humidity arising from the Tisza and Bodrog rivers. The grape varieties are restricted by law to a few white cultivars. The local varieties, Furmint (70%) and Hárslevelű (25%), are complemented by a small percentage of Muscat lunel (a golden-berry mutant of Muscat blanc), Zéta (a local hybrid), and ''Kövérszőlő'' (a recently restored, historical variety of the region).

The climatic conditions, cultivated varieties, and a late harvest favor the development of noble rot almost every year, although the vintage years are not equally good. To support the development of noble rot, very low fruit yields are set in most vineyards.

The harvest in the region is typically very late (from the beginning of October to the end of November). In contrast to several other wine districts, in Tokaj, noble rot is desired to reach where desiccation creates extremely high concentration levels (Fig. 6.1). Optimally botrytized berries (locally called *aszú* berries) are brown, with violet hues, resin-like, and fully shriveled. Hardly any fungal mycelium and conidia are visible or are totally lacking on the surface of the skin. The total extract is above 60°Brix, which corresponds to 500–800 g/l sugar in the juice (Bene, 2004).

FIGURE 6.1 Botrytized grape cluster in Tokaj with aszú (dark) and shriveled (light) berries. Photograph courtesy of Dr. Z. Bene, Tokaj, Hungary.

The juice of the desiccated grapes cannot be extracted by pressing. Thus, a special maceration method has been applied since antiquity, which makes the vinification technology of Tokaji Aszú unique (Alkonyi, 2000; Eperjesi, 2010; Jonson and Robinson, 2001). The two-step process involves selective harvest and storage of the noble-rotted berries; producing a must or base-wine from sound grapes of the same vintage; then soaking and macerating the botrytized fruit in this fermenting must or wine (Commission Regulation, 2009b). The ratio of botrytized fruit to must or wine is indicated on the label by a traditional measure, the *puttony* number, ranging from 3 to 6. The wines fermented from each category must possess a minimum sugar and extract content (60, 90, 120, and 150 g/l sugar and 25, 30, 35, and 40 g/l sugar-free extract, respectively; Hungarian Regulation, 2004).

During the storage of *aszú* grapes, a small part of their juice content seeps out of the berries due to gravity. This is collected under the perforated bottom of the storage container (Alkonyi, 2000). This syrup-like substance is called *Eszencia* or *Essencia* and represents the highest quality Tokaj wine specialty, clearly different from Tokaji Aszú. Fermenting extremely slowly, Tokaji Eszencia has very low alcohol content (typically far below 5%, v/v), but it has enormously high sugar content and fragrance intensity. Extraordinarily sweet and expensive, Eszencia is rarely sold for direct consumption but is rather used for blending the Aszú wines (Eperjesi, 2010). The chemical composition of some Tokaji Eszencia from different vintages is demonstrated in Table 6.3.

Aszú wines must be matured for at least 3 years, during which use of small oak barrels is compulsory for at least 2 years, but longer barrel-aging is preferred by many traditional producers. Different wineries produce Tokaji *Aszú* in remarkably different styles according to the degree of oxidation (Eperjesi, 2010; Kirkland, 1996). This is controlled by the length of barrel-aging and the amount of sulfur dioxide added. However, this never reaches the amount used in Sauternes. In the younger styles, botrytis, quince, raisin, and honey attributes dominate, while walnut, chocolate, and bread flavors develop with age (Robinson, 2006). Tokaji Aszú and Eszencia improve with extended in-bottle aging, perhaps >50 years.

Beside Eszencia and Aszú, Tokaj specialty wines include three more types, Fordítás, Máslás, and Szamorodni (Eperjesi, 2010; Kirkland, 1996). Szamorodni (meaning ''as it was born'') is the most internationally well known. In vineyard sections where the selection of botrytized berries is unprofitable because of their limited presence or adverse weather conditions, noble-rotted and sound berries are harvested together. Vinification follows standard procedures, typical to other white wines. Depending on the initial sugar content of the must, the resulting wine is sweet or dry and is matured for 2 years (at least 1 year in oak barrels).

TABLE 6.3 Modifications of some physical and chemical parameters of grape and juice by noble rot

Reference	Ribéreau-Gayon *et al.* (2000)[a]		Linssen (1986), Dittrich (1989)			Magyar (2006)	
	Sauternes		Germany			Tokaj	
Constituent	Healthy berry	Noble-rotted berry	Healthy berry	Noble-rotted berry BA	Noble-rotted berry TBA	Noble-rotted berry (Essencia)	Noble-rotted berry (Essencia)
Weight per 100 berries (g)	202	98	209	85	36	–	–
Sugar (g/l)	247	317	182	295	500	685	708
Glucose/fructose ratio	–	–	0.98	0.80	0.94	0.98	0.88
Glycerol (g/l)	–	–	0.09	8.00	20.67	24.3	30.7
Total acidity (g/l)	9.23[a]	8.40[a]	11.8	15.2	20.8	16.55	14.7
Tartaric acid (g/l)	5.33	2.48	7.3	2.6	2.4	4.81	4.44
Malic acid (g/l)	5.43	7.84	4.2	8.0	10.1	5.82	7.42
Citric acid (g/l)	0.17	0.22	0.19	0.20	0.24	0.11	0.99
Acetic acid (g/l)	0.32	0.41	0.00	0.45	0.13	–	0.49
Gluconic acid (g/l)	0	2.08	0.02	1.5	2.17	3.20	3.88
Galacturonic acid (g/l)	–	–	0.1	0.6	1.1	–	–
Galactaric acid (g/l)	–	–	0.1	1.0	1.2	–	–
Mannitol (mg/l)	–	–	12	516	2132	–	–
Arabitol (mg/l)	–	–	0	463	818	–	–
Inosit (mg/l)	–	–	148	335	634	–	–
Sorbitol (mg/l)	–	–	30	371	362	–	–
Total polyphenols (mg/l)	–	–	–	–	–	986	1080
Ammonium (mg/l)	85	56	–	–	–	–	–
Amino acids (mg/l)	1282	1417	–	–	–	–	–
Protein (mg/l)	2815	3795	–	–	–	–	–

[a] Concentrations of total acidity organic acids were converted from milliequivalent to gram per litre.

B. German and Austrian botrytized wines

Legend has it that the first German wines produced from noble-rot grapes were unwittingly produced in the vineyards of Schloss Johannisberg in 1775 (Robinson, 2006). This special vintage marked the beginning of intentionally late-harvested botrytized grapes and gave rise to the different styles of botrytized wines in Germany. This ancient wine estate is located in the heart of the Rheingau, in the Rhine valley. It is famous for its excellent Riesling vines. The production of botrytized wines spread throughout the Rhine valley and the Mosel-Saar-Ruwer wine district, although the conditions are not appropriate every year. The special microclimate and the morning mist of the Rhine river occasionally support the noble rot, despite the relatively cold temperatures of the region. Beside Riesling, the predominant cultivars grown, Gewürztraminer, Ruländer (Pinot gris), Scheurebe, Silvaner, and Huxelrebe are prone to noble rot.

Current German regulations, within the *Prädikatswein* category, distinguish three styles that may be or must be produced from noble-rot affected grapes. The minimum grape sugar content for each category is set in Oechsle (Oe) degree, the official measure for grape sugar content in Germany (1 °Oe corresponds to about 5°Brix; see Table 6.1). *Auslese* is made from selected, fully ripe grapes that may or may not be *Botrytis*-concentrated. It can be dry, with high alcohol content, although it is more usually sweet and of low alcohol content. Riesling Auslesen can be some of Germany's most characteristic wines (Robinson, 2006).

According to the official definition (Commission Regulation, 2009b) *Beerenauslese* (BA) is made from specially selected, fully ripe berries with a higher sugar content due to *B. cinerea*. They are harvested later than the designated harvest date. These wines are markedly sweet and have long aging potential. *Trockenbeerenauslese* (TBA) is a highest class of quality wines. They possess special attributes and are made from carefully selected, overripe grapes, whose juice has been concentrated by *B. cinerea*. The berries are shriveled like raisins. The resulting wines offer a lavish sweetness and have low alcohol contents (Commission Regulation, 2009b). The raw material of TBA is partially similar to the *aszú* berries harvested in Tokaj, and the sweetness, extract, and aroma complexity may reach, or rarely exceed those found in six *puttonyos* Tokaji Aszú (Table 6.5). Many Germany vintages yield no TBA wine at all.

The vinification technology of German botrytized wines includes a short maceration of the must on the skins, gentle pressing, and fermentation, which might terminate spontaneously or can be interrupted by sulfite addition and filtration (Dittrich, 1977; Troost, 1980). These wine types generally have low alcohol contents and are rarely matured in oak barrels. Early bottling is typical. The color is golden to deep golden, sometimes deep caramel (Robinson, 2006). The high and fine acidity balances the high sugar content. The wines are characterized by rich flavors, with notes of apricot, honey, caramel, and dried fruit, and by an acidic character much more pronounced than in Sauternes, typically similar to Tokaji.

Similar late-harvest wine categories are recognized by the Austrian wine law as well, although the required sugar contents of the grapes are somewhat higher, because of the warmer climate (Table 6.1). Beside the categories BA and TBA, an additional type called *Ausbruch* exists. It can be made, as an option, by selective picking (breaking out) of the most perfectly noble-rotted berries only, and processing them with maceration similar to Tokaji Aszú. The picking of botrytized grapes has to be announced to the local authorities on the morning of the day of harvest (Austrian Wine Law, 2009). Overripe, naturally shriveled grapes are allowed to be used without *Botrytis* infection, although this is not typical.

The most famous Austrian botrytized wine is Ruster Ausbruch, produced by Lake Neusidel (Burgenland). It is produced primarily from highly botrytized Furmint and Muscadel, but also Pinot Blanc, Pinot Gris, Chardonnay, Neuburger, Traminer, and Welschriesling are used. The humidity derived from the large and shallow lake favors noble rot regularly. The quality of the wines in this region may reach TBA level as well. The wines are normally aged in wooden casks or oak barrels. The length of time and type of barrel used depending upon the style of the vintner. Botrytized wines are produced also in the village Gumpoldskirchen, mainly from the autochthonous varieties Zierfandler and Rotgipfler.

C. Sauternes and other French styles

Produced within the Bordeaux wine district, Sauternes is probably the best known among botrytized wines. Sauternes is located along the Garonne river and its tributary, the Ciron. Unlike other Bordeaux regions, Sauternes is specialized for white, sweet wine production. The meeting of the two rivers, with different water temperatures, regularly generates morning mist, when the autumn is warm and dry. These conditions frequently favor noble rot, although the intentional use of *Botrytis* attacked grapes for sweet wine making began two centuries later than in Tokaj (Jonson and Robinson, 2001).

The area sits on an alluvial plain, with sandy, limey soils (Robinson, 2006). The appellation is reserved for wines from five communes (Barsac, Sauternes, Bommes, Fargues, and Perignac). The present wine classification was introduced in 1855, along with the classification of the red Bordeaux wines. Grape and wine production has recently been regulated in detail (Décret, 2009), including vineyard locations, viticulture practices (varieties, vine density, training system, crop yield, etc.), and some principal quality parameters of the must and wine (Table 6.2). Four grape varieties are planted: Sémillon, Sauvignon blanc, Sauvignon gris, and Muscadelle. Sémillon is the principal grape cultivated. It is especially susceptible to noble rot and accounts for about 80% of a typical estate's vineyard.

The official classification of Bordeaux wines distinguishes three quality categories. In Sauternes, a single winery (Château d'Yquem) belongs to the Premier Cru Supérieur class, 11 estates belong to Premier crus, and 15 to Deuxième Crus. Many wineries are not classified but are entitled to use the Sauternes AOC, or Barsac AOC in Barsac wineries. In poor vintage years, most of the wines are simply labeled Bordeaux AOC. Maximum yields are restricted to 25 hl/ha (1.4 tons/acre; Décret, 2009), but at the higher class estates, the yields probably fluctuates between 12 and 20 hl/ha, and is 9 hl/ha on average at Yquem (Robinson, 2006).

Sauternes from the best locations and in good vintage years have a strong *Botrytis* character, with notes of apricots, honey, and peaches. These are preserved for a very long time in bottle due to the relatively high sulfur dioxide content. Richness of flavor and elegance may have preference over sweetness, body, and acidity in the overall quality of Sauternes. When young, its color is golden yellow, gradually deepening with age. (McCarthy and Ewing-Mulligan, 2001). In exceptional years, the wines are very long lived and thought to improve i'n-bottle for more than 100 years—although this would be difficult to confirm.

Another style of botrytized sweet wine from France involves the category of ''Sélection de Grains Nobles'' (SGN). This legal definition was introduced in 1984 in Alsace, but similar wines are produced also in the Loire valley from grapes of different varieties and sugar contents (Table 6.2).

The required ripeness level is regulated and expressed in terms of sugar content or potential must alcohol content. In Alsace, Gewürztraminer, Pinot gris (with at least 279 g/l sugar content), Riesling, and Muscat (with at least 256 g/l sugar content) are authorized to produce SGN wines. These values are remarkably higher than those that apply to Sauternes (221 g/l). The style and traditions are similar to those of the German BA, although the alcohol content tends to be a bit higher and the sugar content correspondingly a bit lower, particularly for Riesling and Muscat.

The other appellations of SGN wines are Coteaux du Layon, and Coteaux de l'Aubance in the Loire valley, Anjou. Coteaux du Layon produces sweet wine only, and the single variety here is Chenin blanc, a neutral, acidic grape cultivar. It is extremely prone to noble rot. The required initial sugar content of the must is 294 g/l for SGN wines (INAO, 2007). Two individual AOCs within this region are Bonnezeaux and Chaume. Most of these SGN wines are very sweet, in comparison with the other French styles.

D. Newer styles of botrytized sweet wines

The high prestige and superb quality of botrytized wines has inspired many winemakers, both within and exterior to Europe, to encourage noble rot and produce botrytized sweet wines.

Production of botrytized wines, similar to Sauternes style, is increasing in vineyards located in special areas of Australia, New Zealand, South Africa, and California. In Australia, the most famous type is Botrytis Semillon, produced mainly in the Riverina area of New South Wales. The leading brand used to be labeled ''Sauternes,'' but its name was changed to ''Noble One'' in 1990. This followed a bilateral agreement between Australia and EEC, in which Australia agreed to phase out the use of European names on its wine labels. The harvest is generally very late and is made by harvesting everything in one pass, botrytized and sound grapes together. Typically, oak-barrel aging follows fermentation, with use of varying ratios of new barrique and various maturation times (Table 6.2). Generally, less sulfite is added than in Sauternes. Beside Semillon, some other varieties (Sauvignon blanc, Riesling, or Pinot gris) are also used for botrytized wine production in Australia.

In South Africa, botrytized wines are designated ''Noble late harvest.'' They are increasingly produced in the Western Cape, particularly in the Breedekloof Valley, at the foot of Badsberg Mountain. Its warm days and cool nights during autumn support morning mist formation and the development of noble rot. Chenin blanc and Hanepoot varieties are used, and the technology is more or less similar to that of Sauternes. In New Zealand and California, Sauvignon blanc and Sémillon varieties are typical of botrytized wine production.

In California, the environmental conditions typically do not support the development of noble rot. Nonetheless, a few wineries are occasionally able to produce botrytized sweet wines, mainly by vineyard inoculation of grape clusters with *Botrytis* spores.

Other sweet styles, like icewines, are prospective competitors for botrytized wines. They have the advantage that their production is more predictable and controllable, particularly in countries with cool climates. In hot climates, other natural drying methods are used for concentrating grape juice, including overripening and shriveling of healthy grapes on the vine (late-harvest wines), or in the winery after the harvest (straw wines, *passioto* wines). Under exceptional conditions, these dehydration methods may be combined with a partial botrytization. Nevertheless, the particular aroma composition of botrytized wines, coming from noble rot, is not present in these wines.

E. Passito wines

Picolit (also called Piccolit, Piccolito) is an intermediate style between passioto wines and botrytized wines. It is a traditional, local, grape variety of Friuli, North Italy. While the exact origin of the grape is unclear, Picolit was well known during the eighteenth century, being exported to the royal courts of Europe. The grape is difficult to cultivate, but its high sugar and acid content favor its use for dessert wine production. Both late harvest and *passito* styles are made. For *passito* wines, the Picolit grapes are normally harvested in mid-October and then dried to raisins on straw mats before pressing. The late-harvest styles are picked several weeks later, just before the grapes raisin on the vine. This style is occasionally affected by *Botrytis*. After fermentation, the wine is often aged in oak barrels. The entire PDO (protected designation of origin) is Colli Orientali del Friuli Picolit. The official directive (Decreto, 2006) sets the minimal potential alcohol content at 15% (v/v; corresponding to 253 g/l sugar), with a residual extract of 24 g/l in the wine (without regulation of the residual sugar content).

III. NOBLE ROT

A. Infection by *B. cinerea*

B. cinerea is the anamorphic state of the ascosporogenous species *Botryotinia fuckeliana*, a facultative parasitic fungus. It causes serious losses in many crop species worldwide. While occurrence of the teleomorphic stage in nature is extremely rare, the conidial form, *B. cinerea*, is ubiquitous. Its vegetative reproduction is performed by asexual spores called conidia, which are produced on specially modified filaments, termed conidiophores (Fig. 6.2). The general morphological and physiological characteristics of the genus *Botrytis* have been described by Alur (2004).

B. cinerea belongs to the necrotrophic group of pathogens, which kills plant cells in advance of growing hyphae, totally destroying plant structure. This fungus has long been recognized as a highly diverse pathogen,

FIGURE 6.2 Conidiophore and conidia (spores) of *Botrytis cinerea*. Scanning electron micrograph.

with natural variation modulating an extreme range of phenotypes. In contrast to the other *Botrytis* spp., *B. cinerea* is not a host-specific parasite. It has a broad host range, involving more than 200 plants. Known as polyphageous, *B. cinerea* is a species complex, in which distinct populations may be adapted to different hosts (Choquer *et al.*, 2007).

B. cinerea has great importance in viticulture, frequently causing gray rot (bunch rot), and occasionally noble rot. The economic importance of *B. cinerea* has inspired extensive research activity into its genetics, physiology, ecology, and epidemiology, as well as in the field of disease management, but these are beyond the scope of this review. Recently, the genome of *B. cinerea* has been sequenced and available since 2005 (Fillinger *et al.*, 2007). These results and the improvement in molecular genetic tools have opened the way toward a thorough understanding of the biology of *B. cinerea*.

B. cinerea may infect the flowers and leaves of the vine, although the main target of infection is the berry, particularly after véraison. Early infections of the flowers or green tissues of young berries usually remain latent until the ripening period. The fungus becomes active when the acidity and level of antifungal compounds, for example, stilbenes (Langcake, 1981; Langcake and Pryce, 1977) of the berries decline during ripening (Ribéreau-Gayon *et al.*, 2000; Stein and Blaich, 1985). Under moist conditions, most bunch rot can arise from direct, *de novo* infection of the berry by conidia (Donèche, 1993; Jackson, 2008). Preharvest rains, however, can promote bunch rot through a variety of other mechanisms, such as activation of quiescent infections via increased atmospheric humidity and availability of water to the plant through the soil, and increased secondary spread due to these same higher soil moisture levels (Zitter and Wilcox, 2007).

Having very poor cutinolytic activity, *B. cinerea* cannot penetrate easily through intact berry skin, although direct penetration of the cuticle has also been suggested (Coertze *et al.*, 2001; Gindro and Pezet, 1997; Nelson and Amerine, 1956). Most frequently, infection may occur through stigmata (McClellan and Hewitt, 1973; Nair *et al.*, 1988), pedicels (Holz *et al.*, 2003), natural openings like peristomal microfissures (Pucheu-Planté and Mercier, 1983), or wounds (Nair *et al.*, 1988). The relative importance and frequency of different infection pathways are not completely clear. Wounds or natural microfissures in the berry skin had been regarded as a major pathway (Donèche, 1993). However, Coertze and Holz (2002) found that wounds can be infected only by freshly deposited conidia, and only under wet conditions. Conidia landing on the intact grape skin (before wounding) survive for only a short period. Holz *et al.* (2003) suggest that conidia dispersed during early season infections and, residing superficially within the berry-pedicel attachment zone, are a major factor in *B. cinerea* infections.

B. Process and conditions of noble rot

In contrast to bunch rot, few studies have investigated the infection mechanisms of noble rot. Under dry conditions, latent, early infections may play a significant role. However, under moist conditions, new infections, induced by external conidia, seem particularly important (Jackson, 2008; Magyar and Bene, 2006; Pucheu-Planté and Mercier, 1983). Epidermal penetration by germinating conidia seems basically the same as in the case of gray rot (Donèche, 1993). Peristomal microfissures, which form around the stomata as the fruit enlarges, allow grape exudates to escape through the epidermis, providing nutrients for conidial germination (Donèche, 1993; Pucheu-Planté and Mercier, 1983). One of the notable differences between the two situations is the ripe or overripe state of the berry. During maturation, grapes lose most of their physical and chemical defenses (Ribéreau-Gayon *et al.,* 2000). On ripening, the cuticle becomes increasingly disorganized and its thickness diminishes, supporting the formation of micropores and wounds in the epidermis (Fig. 6.3). These produce additional sites for fungal penetration (Magyar and Bene, 2006). Airborne conidia, landing on the grape surface, are able to obtain nutrients from the berry through these openings. Recent findings show that in humid conditions, germination of conidia can be induced by contact with hard hydrophobic surfaces (e.g., the host cuticle), in the absence of nutrients (Leroch *et al.,* 2007). After a few hours, conidia germinate, producing germination tubes that can penetrate the berry (Fig. 6.3A and B). Penetration is not deep, and subsequent hyphal growth progresses parallel to the berry surface, through the hypodermal tissues (Donèche, 1993; Jackson, 2008). During invasion, the fungus synthesizes and releases several hydrolase and oxidase enzymes (e.g., endo- and exopectinases, cellulase, protease, phospholipase, laccase). These enzymes chemically degrade the epidermis and, diffusing into the berry flesh, catalyze drastic changes in the composition of the juice as well. The color of the white berry skin changes to pink, then brownish, and finally chocolate brown. This latter phase is called the *pourry plein* (fully rotted, but not dried) stage in Sauternes (Donéche, 2003). The growing mycelium mechanically breaks through the cuticle, its filaments emerge through the skin, and develop into conidiophores on the surface (Fig. 6.3C and D). This continues until the berry becomes desiccated due to evaporative water loss.

The loss of moisture is of crucial importance in directing infection toward noble rot versus gray rot. Losing its physiological control, the digested, destroyed berry skin lets the berry dehydrate if conditions are dry. Surface mycelia and conidiophores also contribute to fruit dehydration via evaporation. Since vascular connections between the vine and berry cease at full maturity, the evaporated moisture is not replaced and the juice content becomes highly concentrated (Donèche, 1993; Jackson, 2008). The increasing

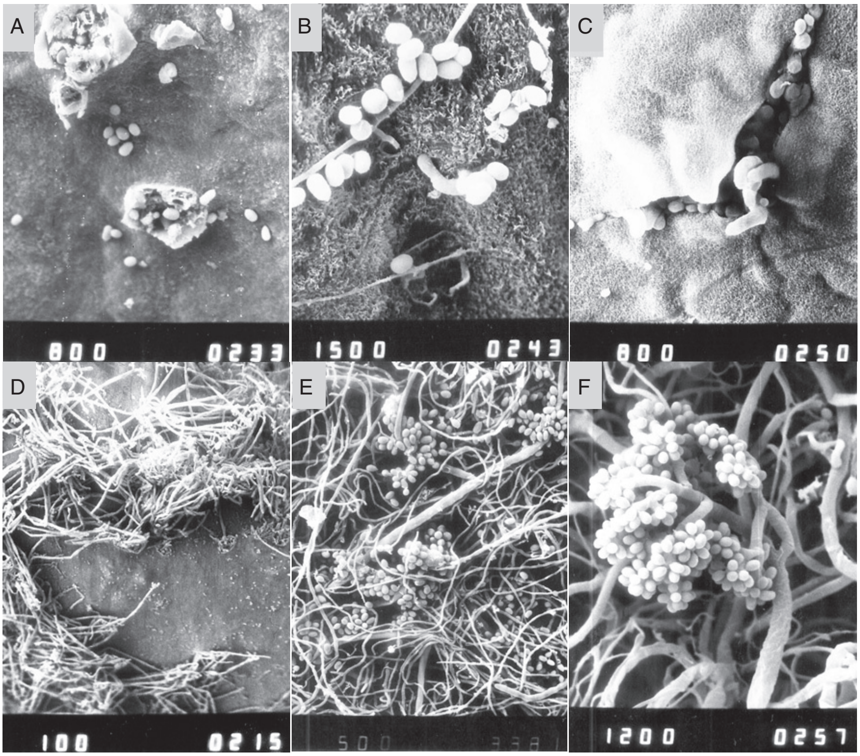

FIGURE 6.3 Invasion of the grape skin by *Botrytis* during noble rot in Tokaj. (A) *Botrytis* conidia on the berry skin which has microinjuries in the cuticle; (B) conidia form germination tube on the epidermal layer of the berry skin; (C, D) the mycelia burst through the cuticle, and come to the surface; (E, F) heavy growth of mycelia and conidia formation on the surface. Scanning electron micrographs. Magnification of the electron optics is indicated at lower left (reprinted from Magyar and Bene, 2006 and with the permission from *Acta Alimentaria*).

sugar content generates increasing osmotic pressure, which limits and modifies further growth and enzymatic activity of *B. cinerea*. The growth of surface mycelia and conidiophores ceases and oxygen uptake by the fungus decreases, further limiting and modifying its enzymatic activities. These phenomena are absent during gray rot, where infection progresses to complete degradation of the berry. The botrytized and shriveled state of the berry is called *pourri roti* in France. It is only in this state that they can be used for sweet wine production in Sauternes, and most other botrytized wine styles in France, Germany, and other countries. In the Tokaj region, the botrytization and concentration processes are expected to reach extremely high levels (Fig. 6.1), producing berries of 60°Brix or higher in total extract (corresponding to 500–800 g/l sugar). Some exceptional TBA wines in

Germany are produced from similar quality grapes; the highest sugar content ever measured in a TBA must was 327 °Oe, corresponding to more than 65°Brix (Dittrich and Grossmann, 2011).

Development of noble rot needs special conditions (Donèche, 1993) that occur in only a few areas in the world. The most important factors are the meso- and microclimatic conditions. Noble rot rarely or never occurs in hot and dry grape-growing areas. Conidial germination is can occur between 10 and 25 °C, although the optimum is 18 °C (Ribéreau-Gayon *et al.*, 2000). Alternating dry and rainy periods are necessary, with primarily dry, sunny days. A short rainy period (3–4 days) just before or at full grape maturity is favorable. An alternating cycle of nighttime humidity, dew, and frequent morning mists or fog favor fungal development, whereas sunny and windy afternoons facilitate water evaporation, limit excessive fungal growth. These weather conditions occur more likely in the late fall, hence late ripening grape varieties are better suited for noble rot development.

Several other varietal properties influence susceptibility to noble rot. Very thin cuticles and the compact grape clusters favor gray rot, whereas thick cuticles resist *Botrytis* attack (Ribéreau-Gayon *et al.*, 2000). High stomatal number, which is variety- or even clone-dependent, favors infection by *Botrytis* (Pucheu-Planté and Leclair, 1990; Pulcheu-Planté and Mercier, 1983).

Grapes respond to fungal attack by producing phytoalexins. These stilbenic derivatives (*trans*-resveratrol, ε-viniferin-dimer, -trimer, -tetramer, pterostilbene) have fungicide properties (Landrault *et al.*, 2002; Langcake, 1981; Langcake and Pryce, 1977; Pont and Pezet, 1990). Phytoalexin production is variety dependent (Landrault *et al.*, 2002, Pucheu-Planté and Leclair, 1990). Correspondingly, a lower capacity for producing phytoalexins favors sensitivity to noble rot. The grape cultivars most commonly used in making botrytized wines are Riesling, Sémillon, Sauvignon blanc, Muscadel, Chenin blanc, Gewürztraminer, Pinot gris, Furmint, and Hárslevelű, but occasionally other varieties may be affected by noble rot.

C. Effects of noble rot on juice composition

Changes in chemical composition and physical properties during noble rot can be summarized as a balance and interaction of the metabolic activity of *B. cinerea* on one side, and the concentrating effect arising from the evaporative loss of water. Both factors are of crucial importance to the quality of noble-rotted grapes. *Botrytis* activity alone leads to rotten, inferior quality grapes, whereas dehydrative concentrating results in only overmatured, shriveled berries. The latter are appropriate for making high quality sweet wines but lack the higher glycerol content and distinctive aroma compounds produced by *Botrytis*.

The main chemical and physical changes during noble rot have long been known (reviewed by Dittrich, 1977, 1989; Dittrich and Grossmann, 2011; Donéche, 1998; Jackson, 2008) and are illustrated in Table 6.3. These latter are based on data concerning Sauternes, German, and Tokaj botrytized grapes. Additional data are provided in Table 6.5.

Berry sugars are utilized by the fungus in producing biomass, energy, and different metabolites. In the young mycelium, glucose is catabolized through the Embden–Meyerhof pathway and the hexose monophosphate shunt. It also possesses an active tricarboxylic acid cycle, and the presence of a glyoxylate cycle have been detected (Donèche, 1989). Direct oxidation of glucose via glucose oxidase leads to gluconic acid accumulation during the stationary growth phase. Developing under the skin, *Botrytis* is in an oxygen-poor atmosphere, which restricts glucose catabolism and hyphal growth. The reduced NAD coenzymes, formed during the oxidative step of glycolysis, are partially regenerated by glycerol-phosphate-dehydrogenase under semianaerobic conditions. Thus, glycerol is also produced during glycolysis, in parallel with the complete oxidation of glucose (Donèche, 1989, 1993). Since glycerol and gluconic acid are practically not found in the juice of sound grapes, they are indicators of *Botrytis* activity (Tables 6.3 and 6.5). However, a significant portion of the gluconic acid content and, in addition, different ketogluconic acids are formed by the acetic acid bacteria that grow on the digested grape skin (Sponholz and Dittrich, 1985). Sponholz *et al.* (2004) suggested that the activity of some wild yeasts also contributes to the elevated glycerol and gluconic acid content of botrytized juice. Gluconic acid is not fermented by yeasts and thus remains unchanged in the finished wine.

Glycerol production is highest during the *pourry plein* stage (Ribéreau-Gayon *et al.*, 2000). Subsequently, it is partially oxidized by the fungus during the external development phase. The terminal glycerol concentration in the *pourris rotis* stage in Sauternes is about 5–7 g/l (Ribéreau-Gayon *et al.*, 2000), but may exceed 30 g/l after further berry dehydration (Dittrich and Grossmann, 2011), for example, in Tokaji *aszú* berries and TBA grapes (Table 6.3 and 6.5). Further, by-products of sugar metabolism include sugar alcohols, like arabitol, mannitol, erythritol (Bertrand *et al.*, 1976). In addition, D-sorbitol and inositol (Dittrich, 1989; Linssen, 1986) accumulate in infected grapes (Table 6.3).

In spite of the significant losses in sugar content due to *Botrytis* metabolism, the sugar concentration of juice increases dramatically, thanks to the concentrating effect of fruit dehydration. Depending on climatic and geographic conditions, grape sugar content can be concentrated by a factor of 2–5 (Ribéreau-Gayon *et al.*, 2000). The final sugar concentration may reach 700–800 g/l in highly shriveled *aszú* and TBA grapes. Because the fungus selectively metabolizes glucose relative to fructose, the G:F ratio of the juice is lower than 1 (50–50%), compared to what is found in ripe, sound berries. The grape sugar composition is also modified via decomposition of grape polysaccharides and pectins by *Botytris* enzymes. These lead to the accumulation of arabinose, rhamnose, galactose, mannose, xylose, and galacturonic acid (Kerényi, 1977; Sponholz and Dittrich, 1985). Galacturonic acid is partially oxidized to galactaric acid (mucic acid). The calcium salt of this acid tends to precipitate in the wine, forming irregular crystals. These are characteristic of botrytized wines in Germany (Würdig, 1976) and Tokaj (Magyar, 2010) but rarely occur in southern wine regions (Ribéreau-Gayon *et al.*, 2000).

The change in acidity during noble rot differs among different varieties and geographic regions. *B. cinerea* utilizes tartaric acid readily (a rare property among microorganisms), but malic acid to a lesser extent (Donèche, 1985). Citric acid is poorly decomposed, or might even be produced by *Botrytis*. The drastic reduction of grape acids is counterbalanced by dehydrative concentration. Depending on the extent of dehydration, juice acidity may fall marginally or rise considerably (Table 6.3).

Beside gluconic, galacturonic, and galactaric acids, *Botrytis* produces small quantities of pyruvic acid and 2-ketoglutaric acid (Dittrich *et al.*, 1974). All these acids, together with those produced by acetic acid bacteria, contribute to higher acidity and a more complex acid composition in botrytized grapes. The typically high acid content is beneficial from the sensory aspect, balancing the extremely high sugar content of some botrytized wines.

B. cinerea utilizes a significant part of the grape's assimilable nitrogen content, including ammonium and amino acids. In some studies, a significant decrease (by 30–80%) in the total amino acid content has been reported in *Botrytis*-affected grapes, compared to the healthy grapes (Dittrich and Sponholz, 1975; Dittrich *et al.*, 1975; Rapp and Reuther, 1971).

It is also postulated that exocellular proteolytic enzymes decompose some grape proteins, liberating nitrogen, amino acids, and smaller peptides. Although many fungal diseases induce synthesis of pathogenesis-related (PR) proteins, particularly before véraison (see Waters and Colby, 2009), the level of PR proteins significantly decreases in grapes or juice infected by *B. cinerea* (Girbau *et al.*, 2004; Marchal *et al.*, 1998), probably due to secretion of fungal proteolytic enzymes.

Conversely, production of exocellular fungal enzymes increases the protein content of the juice, complicating wine clarification and stabilization. According to Ribéreau-Gayon *et al.* (2000), grapes in the *pourry roti* stage contain less ammonium and more complex forms of nitrogen (amino acids and proteins) than musts from healthy grapes (Table 6.4). However, other authors have detected marked reductions in the total amino acid content of *Botrytis*-affected grapes, with significant changes in the qualitative composition (Dittrich and Sponholz, 1975; Rapp and

TABLE 6.4 Effect of *Botrytis cinerea* on some nitrogen compounds of the grape juice

Reference	*n*	Grape	Ammonium (mg/l)	Total amino acid (mg/l)	Proline (mg/l)	Assimilable (N mg/l)	Protein (mg/l)
Dittrich and Sponholz (1975)	12	Healthy	62	3393	509	2884	–
		Gray rot	47	1985	247	1738	–
		Change %	−24	−41	−51	−40	–
Rapp and Reuther (1971)	5	Healthy	44	2719	336	2383	–
		Noble rot	25	1077	79	998	–
		Change %	−43	−60	−76	−58	–
Ribéreau-Gayon *et al.* (2000)	–	Healthy	85	1282	–	–	2815
		Noble rot	56	1417	–	–	3795
		Change %	−34	+10.5	–	–	

n, number of grape varieties examined.

Reuther, 1971). Relative to vitamins, thiamin and pyridoxine contents are seriously reduced in botrytized must (Dittrich and Sponholz, 1975).

In addition to major constituents, *Botrytis* is able to synthesize numerous chemical compounds in small amounts. It produces two different groups of polysaccharides, both of them having oenologic importance. One of these is a pure β-D-glucan (also termed cinerean), consisting of glucose units with β-1,3-linkages in the main chain (Dubourdieu and Ribéreau-Gayon, 1981; Dubourdieu *et al.*, 1978a). Single glucose units are attached to this backbone at approximately every second to third residue of the main chain by β-1,6 linkages. Its molecular weight ranges between 100,000 and 1,000,000 Da. These polysaccharides are neutral from the sensory aspect, but they make strand-like colloids in an alcoholic medium. These linear macromolecules act as protective colloids, making wine clarification difficult. They are particularly disadvantageous during filtration, quickly plugging filter sheets even at very small concentrations (2–3 mg/l; Wucherpfennig and Dietrich, 1983; Wucherpfennig *et al.*, 1984). *B. cinerea* also synthesizes β-D-glucanase enzymes after sugar depletion. This is irrelevant in the high sugar content grapes but has importance in industrial β-D-glucan production (Stahmann *et al.*, 1992).

The second group of *Botrytis* polysaccharides isolated and characterized by Dubourdieu (1978) consists of mannose and galactose, with a small amount of glucose and rhamnose. Their molecules are smaller (20,000–50,000 Da). They may provoke acetic acid and glycerol production of yeasts during fermentation, particularly at the final stage (Donèche, 1993). They may correspond to the inhibitory substance once termed "botryticin" (Dittrich, 1977; Dubourdieu *et al.*, 1978b).

B. cinerea produces an exocellular laccase: *p*-diphenol oxygen oxidoreductase (Dubernet *et al.*, 1977). It can transform the principal white grape phenolics to quinones. Unlike grape tyrosinase, laccase can oxidize a very broad range of phenolic compounds (Salgues *et al.*, 1986). Polymerized quinones form brown compounds, which are probably responsible for the chocolate brown color of the botrytized berry. The increasing sugar concentration during shriveling progressively inhibits the production and activity of laccase, which falls down at the *pourry roti* stage of Sauternes grapes (Donèche, 1993).

In addition to the high sugar and extract content, the main benefit of noble rot lies in the modification of aromatic substances in the juice, and formation of unique odorous derivatives. Terpenols, primary aroma compounds in grapes, are diminished during noble rotting (Schreier *et al.*, 1976). These compounds are liberated from their glycosides by β-glycosidases of fungal and grape origin. The terpenols are subsequently oxidized by fungal enzymes to odorless compounds (Bock *et al.*, 1988; Rapp and Mandery, 1988). This leads to a reduction in varietal aroma, whereas a large number of new aromatic compounds are produced by *Botrytis*.

Most of the studies on *Botrytis*-specific aroma compounds have investigated the aroma composition of wines (Table 6.7). Only a few have focused on what occurs in the grape. On synthetic media, the main odorous compounds produced by *B. cinerea* are aromatic aldehydes (benzaldehyde, phenylacetaldehyde), and furfural (Kikuchi *et al.*, 1983). Sarrazin *et al.* (2007a) confirmed the higher concentration of phenylacetaldehyde in noble-rotted grapes.

Numerous γ- and δ-lactones were identified in Tokaji *aszú* grapes (Miklósy and Kerényi, 2004; Miklósy *et al.*, 2004). The odor notes of the γ-lactones were described as resin- and caramel-like, roasted, or honey, while the δ-lactones exhibited characteristic notes of coconut, chocolate, and peach. The same lactones had been identified earlier from botrytized wines but not from normal wines (Schreier *et al.*, 1976). Lactones are mostly found in oxidatively aged wines but seem to develop in fruit due to the oxidizing effect of *B. cinerea*, water loss, or Maillard reactions (Miklósy *et al.*, 2004).

Sarrazin *et al.* (2007a) established that the development of *B. cinerea* led to an increased concentration of homofuraneol, furaneol, norfuraneol, and phenylacetaldehyde in wines produced from botrytized grapes, in comparison with wines made from healthy grapes.

Recent studies have focused on cysteine-*S*-conjugates as varietal aroma precursors (see Baumes, 2009). Chemically, these odorless compounds are *S*-substituted derivatives of L-cysteine, differing in the attached to sulfur atom. During fermentation, extremely odorous volatile thiols are formed from these precursors (see Dubourdieu and Tominaga, 2009). One of these thiols, 3-sulfanylhexan-1-ol (3SH), is known as an important aroma compound in Sauvignon blanc wines. Its precursor, *S*-3-(hexan-1-ol)-L-cysteine (P-3SH), is found in healthy grapes, but production was considerably amplified when *B. cinerea* infected the grapes. A determination of P-3SH distribution demonstrated that *B. cinerea* was not directly responsible for precursor synthesis, but probably stimulated the grape metabolic pathway involved in its formation (Thibon *et al.*, 2009).

In addition to P-3SH, three new cysteine-*S*-conjugates, *S*-3-(pentan-1-ol)-L-cysteine (P-3SP), *S*-3-(heptan-1-ol)-L-cysteine (P-3SHp), and *S*-3-(2-methyl-butan-1-ol)-L-cysteine (P-2M3SB), have recently been isolated from botrytized grape must. They seem to be specific products associated with noble rot of Sauvignon blanc and Sémillon grapes (Thibon *et al.*, 2010). The thiols formed from these precursors during fermentation are important odor active compounds in their botrytized wines (see Section IV.C).

D. Effects of noble rot on the grape microbiota

By disrupting the grape epidermal layer, *B. cinerea* opens the way for the growth of saprophytic fungi and bacteria. Of these microorganisms, yeasts are the most important from an enologic perspective. Earlier examinations of the population dynamics on the surface of botrytized grapes (Antunovics *et al.*, 2003; Bene and Magyar, 2004; Le Roux *et al.*, 1973; Peynaud and Domercq, 1953; Rosini *et al.*, 1982; Sipiczki *et al.*, 2001) revealed the significant presence of *Candida stellata* (syn. *Torulopsis stellata*) and *Kloeckera apiculata*. Using molecular taxonomic methods, *C. stellata*-like isolates of Tokaj Aszú were found to be significantly different from the type strain of the species. Upon determining the nucleotide sequences in the 26S and 5.8S–ITS regions of the rDNA, Sipiczki (2003, 2004) described the yeast as a novel species, under the name *Candida zemplinina*. Further studies revealed that the *C. stellata* isolates, which had been reported as typical yeasts of botrytized grapes, were most likely to have been strains of *C. zemplinina* strains (Csoma and Sipiczki, 2008). *C. stellata* strains were also isolated from Tokaj wine fermentations (Bánszky *et al.*, 2003) and from botrytized grapes in Tokaj (Magyar and Bene, 2006) but at much lower frequencies. The two sibling species are phenotypically similar (Magyar and Tóth, 2011; Sipiczki, 2004) and can only be differentiated by molecular methods. Both species are sugar-tolerant and cryotolerant (Csoma, 2008; Sipiczki, 2004). This might explain their adaptation to the conditions of noble-rotted grapes. Interestingly, both *Candida* species, as well as *K. apiculata* are fructophilic, unlike the majority of the yeasts (Magyar and Tóth, 2011). Whether or not this property has a role in their prevalence on botrytized grapes is not known. *Metschnikowia pulcherrima* (*C. pulcherrima*) was found as another typical yeast of *aszú* grapes in Tokaj and was predominate yeast when the samples were taken from the vineyard directly (Bene and Magyar, 2004; Magyar and Bene, 2006). Using a different isolation strategy and molecular identification on a high number of random isolates, Csoma (2008) reported similar results. In Tokaj vineyards *H. uvarum (K. apiculata)*, *M. pulcherrima* and its close relative, *M. fructicola*, dominated, followed by *C. zemplinina*, other *Candida* and different basidiomycetes species. The population of *M. pulcherrima* declined after picking and storage, whereas the presence of *C. stellata/zemplinina* and other sugar-tolerant yeasts increased during *aszú* grapes storage (Bene and Magyar, 2004; Magyar and Bene, 2006).

Analyzing the microbiota of fresh must, Fleet *et al.* (1984) also detected a significant population (10^4) of *C. pulcherrima* in botrytized must (Sauternes) but not in healthy must. These data show that *C. zemplinina* and *M. pulcherrima* are strong competitors for *B. cinerea*, although the biochemical rationale is not known. *M. pulcherrima* was found inhibitory to a range of other yeasts, including *S. cerevisiae* (Nguyen and Panon, 1998). Their presence has been suggested as a biocontrol agent against postharvest fungal pathogens (*B. cinerea*, *P. expansum*) on apple (Saravanakumar *et al.*, 2008). Competition for nutrients (e.g., iron) and space is considered to be a primary mode of inhibition (Sipiczki, 2006), although other mechanisms cannot be excluded.

Using direct isolation, without enrichment, *Saccharomyces* species were not found on Tokaj *aszú* berries (Csoma, 2008; Magyar, 2006; Magyar and Bene, 2006), although Naumov *et al.* (2002) reported the presence of *S. uvarum* and *S. cerevisiae* on Tokaj grapes (method of isolation unknown). During spontaneous fermentations, however, diverse *Saccharomyces* populations can be detected in botrytized musts (see Section IV.C).

Development of *Botrytis* grapes, even in the form of noble rot, is always accompanied by the growth of saprophytic fungi. *Penicillium* and *Aspergillus* species are commonly found in widely varying numbers (10^3–10^6 conidia/g berry), depending on the year. Average conidia numbers are one to two times lower than those of *Botrytis* (Bene and Magyar, 2004). Along with *Botrytis*, Kalmár *et al.* (1999) identified six *Aspergillus*, three *Penicillium*, and two *Mucor* species on *aszú* berries. Furthermore, Csoma (2008) reported a significant presence of *Aureobasidium pullulans*. Consistent with the presence of *Penicillium*, an acid-tolerant penicillin derivative, penicillin-V (phenoxy-methyl-penicillin), was detected in varying but generally low concentrations in most Tokaji Aszú wines (Kállay and Bene, 2003).

The population of acetic acid bacteria significantly increases on the botrytized grape, which results in formation of acetic acid and other compounds. In contrast to *Acetobacter* species, *Gluconobacter oxydans* prefers a sugar-rich environment, producing gluconic, 2-ketogluconic, 5-ketogluconic, and 2,5-ketogluconic acids from glucose (Olijve and Kock, 1979; Sponholz and Dittrich, 1985). These ketonic acids are partly responsible for the high SO_2 binding capacity of botrytized must and wines. Only moderate amounts of acetic acid are formed from the oxidation of ethanol, which is present in low concentrations in noble-rotted grapes. The juice extracted from botrytized berries contains a considerable amount of acetic acid, but wild yeast species on the fruit may play a role in its production (Donèche, 1993).

Little is known about the presence and importance of lactic acid bacteria on noble-rotted berries. Fleet *et al.* (1984) detected low numbers (10^2 ml^{-1}) of LAB (mainly *Pediococcus*) in freshly extracted must from botrytized grapes. Their numbers remained low throughout the fermentation. Although the high sugar content would support their growth (Donèche, 1993), the complex nutrient demands and poor competitiveness of these bacteria generally prevent their activity on botrytized grapes or in wines (Magyar, 2010).

E. Induction and control of noble rot

The artificial induction of noble rot would greatly facilitate making botrytized sweet wine, extending their production to countries where conditions are unfavorable for the natural development of noble rot. Experiments have long been performed to this end. In the earliest work, Nelson and Amerine (1956) unsuccessfully tried to induce its development in the vineyard by inoculation. The necessary moisture condition after inoculation was impossible to reproduce under field conditions. In addition, the method creates the risk that other fungi (*Penicillium*, *Aspergillus*, *Rhizopus*), yeasts and, acetic acid bacteria could develop if unfavorable weather conditions arose (Dittrich, 1977).

In later studies, Nelson and Amerine (1957) and Nelson and Nightingale (1959) inoculated harvested grapes with spore suspensions of *B. cinerea*. These were stored on trays under strictly controlled environmental conditions (temperature, humidity) resembling those essential for noble rot development. The same method has been reported to be used in certain regions in Australia, where the climate around harvest time is hot and dry, and *B. cinerea* does not naturally infect grapes (Ewart, 1982). The fruit is harvested, placed on trays, inoculated with a spore suspension and incubated at 90–100% relative humidity at 20–25 °C for 24 h. This period is followed by longer storage under cool, dry conditions to limit the growth of the *Botrytis* and facilitate berry dehydration. The method was promising but has not been adopted significantly due to its expense.

Several attempts were made to inoculate *Botrytis* spores or enzymes directly into juice or must (King *et al.*, 1969; Watanabe and Shimazu, 1976). Field inoculation, however, is closer to the natural noble-rot process and is likely to be more acceptable to consumers. Thus, vineyard experiments are continuing.

In experiments conducted in Burgenland, Austria, it was found that artificial inoculation of ripening berries with a *Botrytis* spore suspension induced only a modest increase of noble rot infestation. Weather condition played a crucial role in the process (Gangl *et al.*, 2004). Field experiments with inoculation of Malvasia grape resulted in inferior wine quality in comparison to postharvest inoculation of grape under controlled conditions (Tiberi *et al.*, 2008/2009). Successful spray inoculation of vineyard with *Botrytis* conidia, however, was reported from ''Dolce'' wine production in Napa Valley, California (Mills *et al.*, 2002). These methods need further research and development into the production of conidia or mycelia for the inoculation step (Akau *et al.*, 2004; King *et al.*, 1969).

IV. PRODUCTION OF BOTRYTIZED WINES

Due to the particular raw material, making botrytized wines is major challenge for winemakers. In addition to the uncertain nature of noble rot development, low grape and juice yield, technological difficulties, and the high risk of spoilage, make producing these wines one of the world's most expensive.

A. Harvest

Various grape-picking strategies and techniques are applied in botrytized wine making. Regardless, a prolonged or late harvest is necessary a factor which implies risk of losing the crop to bunch rot, other infections or frost under adverse weather conditions.

The most labor intensive harvest procedure involves going through the vineyard periodically, picking only individual, perfectly noble-rotted berries, leaving the rest until they reach a shriveled state. This method is generally used in Tokaj for all Aszú wine types (Alkonyi, 2000). The grape clusters are left on the vine until November and finally picked together. Depending on the content of botrytized berries, these grapes are used in making dry wines, sweet late-harvest wines, or Tokaji Szamorodni (Eperjesi, 2010; Kirkland, 1996). Selective harvest of individual berries is also occasionally used in Austria and Germany for making superb quality TBA wines.

Another method involves repeated selective harvest of botrytized grape bunches, or bunch sections, containing berries at different level of noble rot but predominantly in the *pourri rotis* stage. This method, called *triage*, is the characteristic of Sauternes. Climatic conditions dictate the number of selective pickings per year—up to three or four (Ribéreau-Gayon *et al.*, 2000). A typical harvesting pattern involves picking half the botrytized Sauvignon grapes in late September, then in late October picking the Sémillon and remaining Sauvignon grapes in subsequent *triages* over a 3-week period in November. During this period, gray-rotted berries are eliminated (Robinson, 2006). Selective harvesting of *Botrytis*-

affected grape clusters is also widely used in other traditional botrytized wine types (e.g., SGN, Ausbruch, BA, most TBA).

The less labor intensive, but still expensive, harvest method involves waiting for as long as possible, and then picking the botrytized and healthy grapes together as whole bunches. This method is used all over the world in the making new-style botrytized wines.

Due to the special technology of Tokaji *Aszú*, the selected aszú berries are exposed to a postharvest operation and storage, which is unique in winemaking. The collected berries are transported to the winery and stored in small containers, generally for several weeks. During this time, an autoselection process occurs in the saprophytic mycobiota of *aszú* grapes due to the selective pressure of the special microecologic conditions (Bene and Magyar, 2004; Magyar and Bene, 2006). Populations of *M. pulcherrima* and *H. uvarum* prevailing on the *aszú* berries in the vineyard decline, and *C. zemplinina* becomes dominant. Other sugar-tolerant, fermentative species, like *Zygosaccharomyces*, *Torulaspora*, and *Kluyveromyces* became more pronounced as well. The optimal storage conditions for control of the desirable and undesirable species on *aszú* berries have been studied (Tóth *et al.*, 2007) but need further research.

B. Grape processing

Botrytized grapes need to be manipulated with particular care, to avoid physical damage to the grape skin, formation of suspended solids (vegetal tastes), and diffusion of excess glucan into the juice (Ribéreau-Gayon *et al.*, 2000; Troost, 1980). In Sauternes, the grapes are crushed, but generally not stemmed, to facilitate the drainage of juice during pressing. Soaking of the gently crushed fruit overnight in its own juice is general practice in many regions, allowing release of extract and aroma substances (Dubourdieu, 1999; Troost, 1980). Oxidation is limited by using closed vessels or a layer of CO_2 gas. Free-run juice cannot be separated because of the high viscosity of the must (Ribéreau-Gayon *et al.*, 2000). Juice extraction is extremely difficult, needing slow pressing in two to three, or more, repeated cycles. Great pressure must be exerted on the grapes to extract the vacuolar content of cells (Donèche, 1993). Standard pneumatic presses are not sufficient because of their low pressing strength. Continuous screw presses, on the other hand, are too drastic and should not be used (Ribéreau-Gayon *et al.*, 2000). Unlike healthy grapes, juice obtained from the second and subsequent press cycles contains more sugar and extract and has high quality.

To make juice extraction easier, a cold pressing (cryoextraction) technology has been developed (Chauvet *et al.*, 1986). Cooling the grapes below 0 °C (potentially as low as −16 °C) freezes berries with lower sugar contents, while the juice of berries with the highest sugar content remains in liquid form. This permits selective juice extraction during pressing. By this method the richest juice fraction can be isolated, making it possible to produce highly concentrated sweet wines in poorer vintage (Dubourdieu, 1999).

Juice extraction for Tokaji Aszú production is completely different. The Commission Regulation (2009b) defines *Aszú* as a wine made by pouring new wine, must, or fermenting must onto botrytized (*aszú*) berries. The botrytized berries are stored in the winery, while the healthy or mixed grapes are vinified by normal methods to make a base juice or wine. The most characteristic step of *Aszú* making is the maceration procedure, where different ratios of gently crushed botrytized grapes (paste), or occasionally uncrushed berries, are added to the base juice or wine. This ratio is indicated by the *puttony* number on the label, ranging from 3 to 6. *Puttony* is a traditional hod of 20–22 kg capacity (Kállay, 2005). One *puttonyos aszú* would be made by the maceration of one *puttony* of noble-rotted berries with 136 l of base juice (the volume of a traditional barrel). The lowest *puttony* number is three. Today, common measures (20 kg *aszú* grapes to 100 l must or wine) are used. Thus, a 5 *puttonyos* Aszú is made from a mixture of 100 kg of *aszú* berries and 100 kg of juice or wine (Alkonyi, 2000; Eperjesi, 2010).

The length (24–48 h) and technique (open vats, tank presses, rototanks) of maceration vary from winery to winery. During maceration, cell wall degradation is completed, and the sugar, extract, and aroma compounds diffuse into the juice. Extraction of the juice occurs without marked difficulty (drainage of free-run juice, and gentle pressing). Since the extracting liquid used for soaking the *aszú* grape is generally new wine or partially fermented must, the juice obtained after maceration and pressing (*aszú*-base, or raw *aszú*) normally has significant alcohol content, unlike other botrytized styles (Magyar, 2010).

A slight sulfiting of the must (3 g/hl) is favorable for selecting microorganisms and assuring the development of favorable yeasts (Dubourdieu, 1999; Ribéreau-Gayon *et al.*, 2000).

Juice clarification before fermentation is widely applied in most botrytized wine technologies. However, this step also has unique difficulties in comparison with normal musts, due to the presence of *Botrytis*-derived polysaccharides. Pectolytic enzymes that hydrolyze α-glycosidic bounds are ineffective on the β-glucans of *Botrytis*, but commercial *Trichoderma* β-glucanases are available (Dubourdieu *et al.*, 1985; Villettaz, 1990; Villettaz *et al.*, 1984) and have been authorized. Nonetheless, due to its expense glucanases are seldom used (see Section IV.D). Clarification usually involves simple settling. Decanting occurs 18–24 h after pressing when the heavier particles have settled (Dubourdieu, 1999). At low temperatures (0 °C), settling time can be extended to 2–3 days, permitting more effective clarification. Excessive clarification is not desired, since it may accentuate any nutrient deficiencies already present in the must. Bentonite treatment is typically not employed (Ribéreau-Gayon *et al.*, 2000). However, Dittrich and Grossmann (2011) emphasize the need for effective clarification of botrytized, press-run juice using advanced techniques.

Depending on the local legislation in France, Germany, and the New World countries, various adjustments such as sugar addition and acid correction can be made to the juice (Ribéreau-Gayon *et al.*, 2000) although these are rare. Sugar addition, either in the form of chaptalization or preserved must, is forbidden for Tokaji, French SGN, and Austrian and South African botrytized wines.

C. Fermentation

1. Yeasts

Fermentation of botrytized musts is a slow process. It may take 1–6 months, 1 year not being exceptional. These musts possess particular initial yeast biota and provide extremely difficult nutritional and environmental conditions for yeasts.

The population dynamics of yeasts during spontaneous fermentation of botrytized wines has been thoroughly studied, for example, in Bordeaux wines (Fleet *et al.*, 1984; Ribéreau-Gayon *et al.*, 1975), in Tokaji Aszú (Antunovics *et al.*, 2003; Magyar *et al.*, 1999; Minárik and Laho, 1962; Sipiczki *et al.*, 2001) in Californian wines (Mills *et al.*, 2002), and in Greek wines (Nisiotou *et al.*, 2007). In accordance with the complex yeast biota of botrytized grapes, these wines display a higher level of biodiversity than normal wines. Presumed *C. stellata* (probably *C. zemplinina*) and *C. zemplinina* strains dominate fermentation during the first weeks in most cases (Fig. 6.4). They also survived long after *Saccharomyces* strains began to dominate, particularly at lower temperatures (Fleet *et al.*, 1984; Magyar *et al.*, 1999; Mills *et al.*, 2002; Nisiotou *et al.*, 2007). The other genera most frequently isolated from the mid-fermentation stage are *Kluyveromyces*, *Zygosaccharomyces*, *Hanseniaspora*, and *Pichia*. Mills *et al.* (2002) reported large populations (10^6 cells/ml) of a viable but nonculturable (VBNC) *Candida* strain (later identified as *C. zemplinina* by Sipiczki, 2003) from botrytized wine fermented at higher temperatures and also a VBNC *Hanseniaspora* strain survived for long period.

These results suggest that non-*Saccharomyces* species may contribute significantly to the fermentation of botrytized wines. *C. zemplinina* seems not to produce excess volatile compounds nor any specific aroma compounds (Tóth-Márkus *et al.*, 2002). Its main contribution to the chemical composition might be an increase in glycerol content and in the G:F ratio. *C. zemplinina* and *C. stellata* have proven to be very fructophilic yeasts (Mills *et al.*, 2002; Magyar and Tóth, 2011; Magyar *et al.*, 2008).

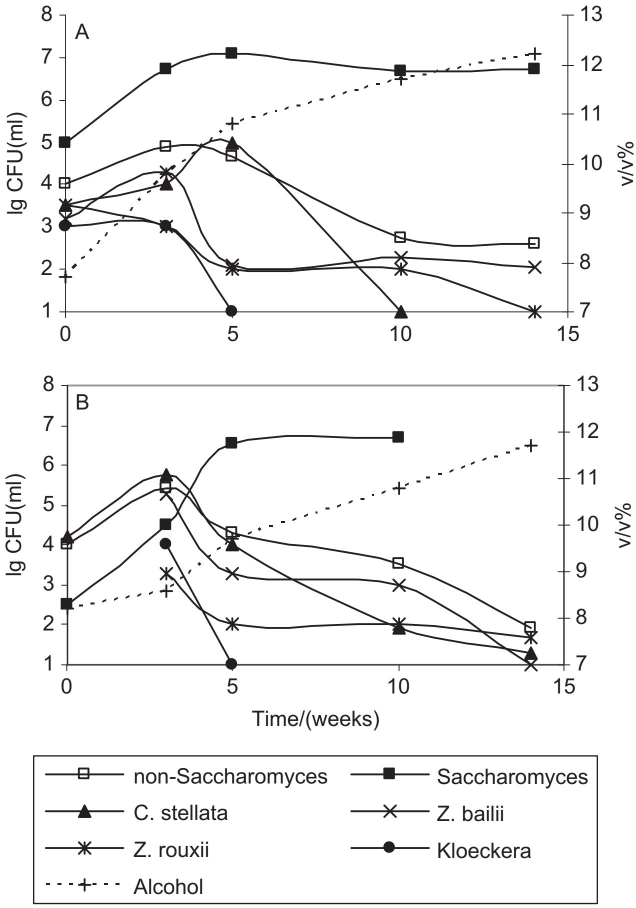

FIGURE 6.4 Course of alcoholic fermentation and evolution of the yeast populations during spontaneous fermentation of Tokaji Aszú. Botrytized berries were macerated with fermenting must (A) or dry wine (B) (Magyar, 2010).

With a few exceptions (Nisiotou *et al.*, 2007), *Saccharomyces* strains dominate fermentation sooner or later, as in nonbotrytized wine. A special feature of botrytized fermentations is that, beside various *S. cerevisiae* races, *S. uvarum* (formerly known as *S. bayanus* var. *uvarum*) is typically isolated from these wines (Antunovics *et al.*, 2003; Magyar *et al.*, 2008; Minárik and Laho, 1962; Naumov *et al.*, 2000, 2002; Sipiczki *et al.*, 2001; Tosi *et al.*, 2009). This species seems to be well adapted to sweet wine fermentations, particularly, but not exclusively, in cooler climates.

S. uvarum is characterized by several authors as a cryotolerant yeast, showing good growth and fermentation rate at low temperatures (7–13 °C; e.g., Castellari *et al.*, 1994; Kishimoto and Goto, 1995).

This yeast, under different species names, has been the focus of several enologic studies because its technological traits are different from those of *S. cerevisiae*.

Recent advances in yeast taxonomy concerning *Saccharomyces sensu stricto* (Raineri *et al.*, 2003; Sipicki, 2002) make it difficult to compare modern data and older research. Taxon names were used inconsistently for the same species, such as *S. cerevisiae p.r. uvarum* (Castellari *et al.*, 1994; Giudici *et al.*, 1995); *S. bayanus* (Kishimoto and Goto, 1995; Kishimoto *et al.*, 1993; Magyar *et al.*, 2008; Sipiczki *et al.*, 2001; Torriani *et al.*, 1999); *S. bayanus var. uvarum* (Naumov *et al.*, 2002); and *S. uvarum* (Tosi *et al.*, 2009; Magyar-Tóth, 2011; Masneuf-Pomarède *et al.*, 2010). *S. uvarum* as an individual species name was suggested by Pulvirenti *et al.* (2000), and is more and more accepted currently, including the subsequent part of the present review.

Different molecular identification methods have been described and used for differentiation of the three phenotypically similar species (*S. cerevisiae*, *S. uvarum*, and *S. bayanus*). These include karyotyping, PCR-RFLP of the *MET2* gene, and microsatellite multilocus typing. None of them seem to be perfect alone, and some phenotypic traits need to be assessed for clear distinction (Antunovics *et al.*, 2005).

According to enologic studies, *S. uvarum* strains ferment more slowly, generate less ethanol, and produce more glycerol and succinic acid, but somewhat less acetic acid, than *S. cerevisiae* (Castellari *et al.*, 1994; Giudici *et al.*, 1995; Magyar *et al.*, 2008; Tosi *et al.*, 2009). Concerning volatile compounds, *S. uvarum* produces several times more 2-phenylethanol and its esters (Bertolini *et al.*, 1996; Massoutier *et al.*, 1998). Masneuf-Pomarède *et al.* (2010) have recently reported a biometric study on 28 *S. uvarum* strains (called *S. bayanus var. uvarum*) isolated from various geographic regions (Sancerre, Jurançon, Sauternes, Alsace, and Tokaj), in comparison with several *S. cerevisiae* strains. Using model juice, they confirmed low ethanol tolerance at 24 °C and production of high levels of 2-phenylethanol and its acetates in *S. uvarum*. They considered these features discriminative. Low acetic acid production was not confirmed as a species specific property (Fig. 6.5). Acetic acid and glycerol production of *S. uvarum* seem to depend on the nutrient medium (Magyar-Tóth, 2011; Magyar *et al.*, 2008). Using *S. uvarum* as starter culture, either alone or in combination with *S. cerevisiae*, may have value in fine tuning and balancing the chemical composition of both normal and particularly botrytized sweet wines. The latter seem to be a special ecological niche for *S. uvarum*. Developing starter cultures from intentional (Kishimoto, 1994) or indigenous (Le Jeune *et al.*, 2007) hybrids of *S. cerevisiae* and *S. uvarum* is another possibility.

The use of selected yeast starters for botrytized wine fermentation is strongly encouraged in Germany (Dittrich, 1977; Hoersch and Schlotter, 1990), in Sauternes (Dubourdieu, 1999; Ribéreau-Gayon *et al.*, 2000), and is typical in the newer botrytized wines produced in Australia, and South

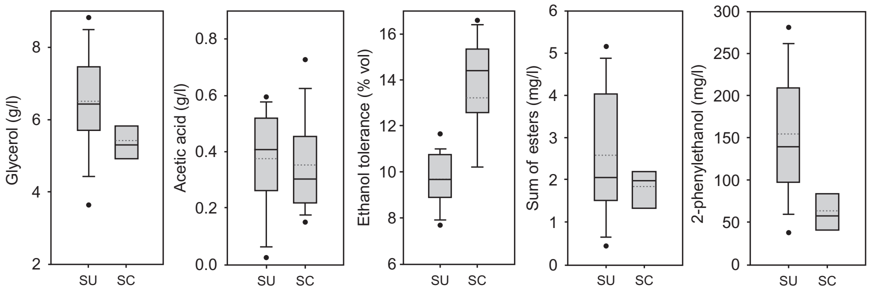

FIGURE 6.5 Box plot analysis for comparison of technological traits in *Saccharomyces bayanus var. uvarum* (28 strains) and *S. cerevisiae* (the number of strains tested was six for glycerol, ester, and 2-phenylethanol production, 65 for acetic acid production, and 71 for ethanol tolerance). Fermentation experiments were carried out in synthetic grape juice of 210 g/l sugar content, at 24 °C. Ethanol tolerance was tested in the same medium containing 300 g/l sugar. (Adapted from Masneuf-Pomarède *et al.*, 2010 and with permission of Elsevier.)

Africa and California. Highly alcohol tolerant and sugar-tolerant strains of *S. cerevisiae var. bayanus* (not identical with *S. bayanus* and *S. uvarum* discussed above) had been generally used for this purpose. However, this physiological race proved to be more sensitive to the inhibitory and acidogenic effects of the heteropolysaccharides of *Botrytis* than *S. cerevisiae var. cerevisiae*. Thus, alcohol tolerant strains of *S. cerevisiae var. cerevisiae* are more preferable (Donèche, 1993).

In Tokaj, most small wineries rely on spontaneous fermentation, although the use of starter cultures is spreading, and is standard in most new estates. Beside commercial starters, some wineries use local *S. cerevisiae* (*var. bayanus*) strains selected from the winery microbiota (Magyar, 2010).

2. Chemical composition

The unique chemical composition of botrytized must greatly impacts the products and by-products of alcoholic fermentation, as well as subsequent reactions. The changes have been extensively studied by German and French authors and have been reviewed by Dittrich (1977, 1989), Jackson (2008), Ribéreau-Gayon *et al.* (2000), and Dittrich and Grossmann (2011). The chemical composition of some traditional (German and Hungarian) botrytized wine styles are illustrated in Table 6.5.

The high sugar content of the juice dramatically reduces the growth, fermentation rate, and survival of yeasts (Dittrich, 1977; Lafon-Lafourcade, 1983) and also affects secondary metabolism. Acetic acid

TABLE 6.5 Chemical composition of some traditional botrytized wines from France, Germany, and Hungary

Wine type	Specific gravity (g/cm^3)	Sugar-free extract (g/l)	Reducing sugar (g/l)	Alcohol (%, v/v)	Total acidity (g/l)	Glycerol (g/l)	Gluconic acid (g/l)	Volatile acidity (g/l)	pH	Reference
Sauternes 1980	–	44	55	– (>13)[a]	5.6[b]	–	1.29[b]	0.75[b]	3.73	Chauvet and Sudraud (1982)
Sauternes 1980	–	52	79	– (>13)	5.4	–	1.00	0.9	3.66	Chauvet and Sudraud (1982)
Sauternes 1980	–	36	148	– (>13)	5.9	–	1.45	1.0	3.84	Chauvet and Sudraud (1982)
Sauternes 1980	–	35	116	– (>13)	5.7	–	1.7	0.8	3.70	Chauvet and Sudraud (1982)
Sauternes, Premiere Cru, 1983	–	–	115	14.7	8.1	–	–	0.78	3.50	Croser, 1989
Sauternes Premiere Grand Cru, 1980	–	–	96.5	13.3	7.7	–	–	1.1	3.50	Croser (1989)
Auslese (mean), Riesling Mosel-Saar-Ruwer, 1976	1.0209	32	57	9.6	7.3	11.4	–	0.6	3.2	Würdig and Woller (1989)
Beerenauslese (mean) Riesling Mosel-Saar-Ruwer 1976	1.0529	45	125	8.7	7.7	15.4	–	0.7	3.5	Würdig and Woller (1989)
Beerenauslese Scheurebe Baden 1976	1.0492	40	123	10.0	6.9	12.7	–	0.7	3.8	Würdig and Woller (1989)

Beerenauslese Riesling Nahe, 1976	1.0597	46	138	7.6	8.9	14.7	–	1.0	–	Würdig and Woller (1989)
Trockenbeerenauslese (mean) Mosel-Saar-Ruwer 1976	1.0640	46	157	10.0	6.6	16.0	–	0.8	3.4	Würdig and Woller (1989)
Trockenbeerenauslese Rulander, Baden 1976	1.1148	76	249	7.3	5.8	24.0	–	1.6	3.8	Würdig and Woller (1989)
Tokaji Aszú 3 puttonyos 2002	–	–	68	13.92	9.3	28.1	2.4	0.6	3.29	Kállay (2003)
Tokaji Aszú 5 puttonyos 1996	–	–	124	12.45	9.8	22.5	2.95	–		Magyar (1998)
Tokaji Aszú 5p uttonyos. 1963	1.0433	36	125	14.41	7.0	21.4	2.68	1.0	3.42	Kerényi (1977)
Tokaji Aszú 6 puttonyos 1973	1.0673	39	187	14.73	8.6	19.3	1.71	0.7	3.58	Kerényi (1977)
Tokaji Essencia 1972	1.2492	112	558	2.58	14.5	35.8	2.67	0.7	3.2	Kerényi (1977)
Tokaji Essencia 1999	1.2930	–	575	1.22	21.8	29.5	5.04	1.11		Magyar (2006)

[a] For Sauternes, no measured data are given. Value in parentheses corresponds to the legal limit before 2009.
[b] For Sauterenes, the values are converted from milliequivalent.

and glycerol production is provoked (Lafon-Lafourcade, 1983). During fermentation, glycerol content, although marked, increases proportionally less in botrytized than nonbotrytized wines (Dittrich *et al.*, 1974).

The stimulating effect of high sugar contents on volatile acidity is expressed in all wines, but it is particularly marked in botrytized wines by the heteropolysaccharides of *Botrytis* (Ribéreau-Gayon *et al.*, 1979). yeast- generated acetic acid production, combined with that generated by acetic acid bacteria on the grape and in the barrel-aged wine, results in an elevated volatile acidity in botrytized wines. This may reach the 2 g/l level permitted by law in some situations.

To limit this possibility, *Torulaspora delbrueckii*, in mixed or sequential culture with *S. cerevisiae*, has been proposed for fermentation (Bely *et al.*, 2008; Ciani *et al.*, 2006; Lafon-Lafourcade *et al.*, 1981; Renault *et al.*, 2009). Although this yeast generates little alcohol, its application in mixed starter cultures is promising. It may also reduce the accumulation of other undesirable by-products, such as acetaldehyde, ethyl acetate, and acetoin.

Production of carbonyl compounds increases dramatically in botrytized fermentations, compared to normal musts. Acetaldehyde, pyruvic acid, and 2-ketoglutaric acid content may be 60%, 350%, and 500% higher, respectively (Dittrich *et al.*, 1975). These compounds accumulated due to the thiamine depletion in botrytized grapes. This limits the decarboxylation of these keto-acids by decarboxylase enzymes (Dittrich *et al.*, 1975). These compounds, along with ketogluconic acids produced by *Botrytis* and *Gluconobacter* (Sponholz and Dittrich, 1985), are responsible for the high SO_2 binding capacity of botrytized wines. Addition of thiamine (0.5–0.6 mg/l) to must is recommended to improve yeast decarboxylase activity and reduce need for SO_2 addition (Dittrich *et al.*, 1975; Dubourdieu, 1999; Hoersch and Schlotter, 1990).

Nitrogen deficiency in botrytized must contributes to slow yeast growth and fermentation rate and to the enhanced production of acetic acid. This can be partially offset by the addition of diammonium phosphate (300 mg/l) or complex nitrogen nutrients. The altered juice amino acid composition influences the production of higher alcohols (Dittrich and Sponholz, 1975), although this also strongly depends on the yeast species and strains dominating during fermentation (Bertolini *et al.*, 1996; Massoutier *et al.*, 1998).

The aroma of botrytized wines has been studied more extensively than that of the grapes. Mashuda *et al.* (1984) identified the lactone sotolon (4,5-dimethyl-3-hydroxy-2,(5)-furanone) as a principal compound in a botrytized aroma. Sotolon is also a key aroma compound in *flor* wines, for example, vin jaune, sherry (Dubois *et al.*, 1976; Martin and Etievant, 1991; Moreno *et al.*, 2005). However, Sponholz and Hühn (1993) found no correlation between the degree of *Botrytis* infection and sotolon concentration—all samples containing low amounts. They concluded that sotolon should not be considered an indicator of *Botrytis* infection. Sotolon is found in botrytized and other long-matured wines, since it can form during aging via a Maillard-type reaction.

The main aroma compounds identified as specific botrytized odorants are indicated in Table 6.6. It seems that while the terpene content decreases, numerous hydroxy-, oxo-, and dicarboxylic acid esters, acetals, and lactones form, all typically in lower concentrations or absent in normal wines (Miklósy and Kerényi, 2004; Miklósy *et al.*, 2000, 2004; Schreier *et al.*, 1976).

In addition to GC–MS, recent studies have focused on the identification and quantitative analysis of impact odorants in botrytized wines using gas chromatography–olfactometry (GC-O) analysis. Sarrazin *et al.* (2007a) investigated numerous botrytized and nonbotrytized Sauternes wines. They could identify several key odorants that were responsible for the sensory differences between the wines, notably 3-mercaptohexan-1-ol, various furanons, ethyl-hexanoate, methional, phenylethanol, phenylacetaldehyde, sotolon, β-damascenone, and 2-methyl-3-furanthiol.

The GC–O methods have revealed the importance of volatile thiols to a botrytized aroma. For example, Sauternes contain a much higher concentration of 3SH than equivalent dry wines made from Sauvignon blanc grapes (Tominaga *et al.*, 2000, 2006). In addition to 3SH (resembling grapefruit and passion fruit), three new specific volatile thiols (3-sulfanylpentan-1-ol, 3-sulfanylheptan-1-ol, and 2-methyl-3-sulfanylbutan-1-ol) were identified in Sauternes wines (Sarrazin *et al.*, 2007b). Their cysteine-*S*-conjugate precursors have recently been identified in botrytized grapes (Thibon *et al.*, 2010; see Section III.C).

The amounts of thiols formed during alcoholic fermentation are strongly affected by the previous development of *B. cinerea* (Table 6.7). Since these compounds have extremely low sensory thresholds, and seem remarkably stable in wine, 3SH particularly plays a significant role in the fruity aroma of botrytized wines (Dubourdieu and Tominaga, 2009).

In a more recent study, Bailly *et al.* (2009) investigated the stability of key odorants during bottle aging in Sauternes wines. Except for 3SH, polyfunctional thiols were found unstable. However, most other key odorants (e.g., sotolon, phenylethanol, esters, γ-lactones, β-damascenone, etc.) were still detected within 5–6 years.

In spite of significant advances, this area needs significant 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.

TABLE 6.6 Volatile compounds associated with botrytized wines as characteristic aroma substances

Compound	Sensory descriptor	Wine type	Reference
Nerol, geraniol and linalool	Orange flower	Sweet Fiano	Genovese *et al.* (2007)
Phenylacetaldehyde	Honey	Sauternes	Sarrazin *et al.* (2007a)
2-Phenyletanol	Rose, floral	Sauternes	Sarrazin *et al.* (2007a), Genovese *et al.* (2007)
3-Mercaptohexan-1-ol	Grapefruit	Sauternes	Tominaga *et al.* (2000), Sarrazin *et al.* (2007a)
Benzyl alcohol	–	Sweet Fiano	Genovese *et al.* (2007)
Ethylhexaonate	Pineapple, green apple, banana	Sauternes	Sarrazin *et al.* (2007a), Bailly *et al.* (2009)
β-Damascenone	Fruity, quince, canned apple	Sauternes Sweet Fiano	Bailly *et al.* (2009), Genovese *et al.* (2007), Sarrazin *et al.* (2007a)
Vitispirane	Champhor	Sweet Fiano	Genovese *et al.* (2007)
γ-Nonalactone	Peach, apricot	Sauternes Sweet Fiano Tokaji Aszú	Bailly *et al.* (2009), Genovese *et al.* (2007), Miklósy *et al.* (2000, 2004), Sarrazin *et al.* (2007a)
δ-Decalactone	Coconut	Tokaji Aszú Sweet Fiano Sauternes	Schreier *et al.* (1976), Miklósy *et al.* (2000, 2004), Genovese *et al.* (2007), Sarrazin *et al.* (2007a)

γ-Decalactone	Peach, apricot	Tokaji Aszú Sweet Fiano Sauternes	Bailly *et al.* (2009), Shreier *et al.* (1976), Miklósy *et al.* (2000, 2004), Genovese *et al.* (2007), Sarrazin *et al.* (2007a)
1-Octen-3-ol	Mushroom	Sweet Fiano	Genovese *et al.* (2007)
Homofuraneol	Caramel, cotton candy, sweet	Sauternes	Bailly *et al.* (2009), Sarrazin *et al.* (2007a)
Furaneol	Caramel	Sauternes	Bailly *et al.* (2009), Sarrazin *et al.* (2007a)
Norfuranol	Caramel	Sauternes	Sarrazin *et al.* (2007a)
Sotolon	Caramell, curry, nut	Sauternes	Sarrazin *et al.* (2007b), Mashuda *et al.* (1984), Sarrazin *et al.* (2007a)
Methional	Baked potatoes	Sauternes	Sarrazin *et al.* (2007a)
3-Sulfanilhexan-1-ol	Fruity, rubarb, grapefruit	Sauternes	Bailly *et al.* (2009), Sarrazin *et al.* (2007b), Tominaga *et al.* (2000)
3-Sulfanylpentan-1-ol	Grapefruit	Sauternes	Sarrazin *et al.* (2007b)
3-Sulfanylheptan-1-ol	Citrus	Sauternes	Sarrazin *et al.* (2007b)
2-Methyl-3-sulfanylbutan-1-ol	Raw onion	Sauternes	Sarrazin *et al.* (2007b)

TABLE 6.7 Assay of volatile thiols (ng/l) in young Bordeaux wines made from grapes at different stages of noble rot, as well as in some bottled Sauternes wines. Adapted from Sarrazin *et al.* (2007b) and with permission from the American Chemical Society

Variety	Botrytis stage	Change of mean grape volume (%)	3SH	3SPOH	3SHpOH
Semillon	Healthy	100	195	traces	traces
	Pourri plein	80	2326	93	34
	Pourri roti	44	3678	124	50
	Late pourri roti	45	6334	291	118
Sauvignon	Healthy	100	161	traces	traces
	Pourri plein	67	3003	141	95
	Pourri roti	27	9648	348	263
	Late pourri roti	37	9319	375	258
Appellation	Vintage	–			
Sauternes	2001		7033	299	63
Barsac	2001		5034	223	44
Loupiac	2002		4749	235	72
Sauternes	2003		5386	199	44

3SH, 3-sulfanylhexan-1-ol.
3SPOH, 3-sulfanylpentan-1-ol.
3SHpOH, 3-sulfanylheptan-1-ol.

3. Fermentation technique

The fermentors used in most regions are stainless steel tanks, although wooden barrels are still widely used in Sauternes and Tokaj. The addition of thiamin (0.6 mg/l), diammonium phosphate (300 mg/l), and active dry yeast (10–15 g/hl) is recommended to achieve an optimal fermentation rate, more rapid yeast propagation and reduce SO_2 requirements (Dubourdieu, 1999; Hoersch and Schlotter, 1990).

There are no general rules for temperature control in botrytized wine fermentations. During in-barrel fermentations, the temperature can easily reach 28 °C in Sauternes (Donèche, 1993). In traditional cellars in Tokaj, barrel fermentation is occurs at 10–12 °C. Although making the process more difficult, it may explain the high presence of cryotolerant *S. uvarum*. Due to the very late vintage, low fermentation rate, and use of small barrels (200–230 l), Tokaj Aszú fermentation does not require cooling. Conversely, heating would be beneficial in many cases. In large fermentation tanks, most wineries keep fermentation temperatures around 20 °C in Tokaj (Magyar, 2010) and between 20 and 24 °C in Sauternes (Ribéreau-Gayon *et al.*, 2000).

A special aspect of botrytized wine making is the cessation of fermentation at a desired residual sugar content. Traditionally, fermentation stops spontaneously at various ethanol levels, which sometimes were much higher than desirable, leading to insufficient residual sugar.

This can be prevented by artificial cessation of fermentation. Pasteurization would be effective but is not widely used due to aroma considerations. The most frequent technique in Sauternes, Germany, and many other wine regions is by the addition of sulfur dioxide. The presence of at least 50 mg/l free sulfite is necessary to terminate fermentation (Donèche, 1993). To provide this level, a large amount of SO_2 has to be used, since most of the added sulfite combine with the carbonyl compounds, notably keto-acids. Typically, the addition of 200–300 mg/l SO_2 is required to achieve the necessary 60 mg/l free SO_2 level. This level needs to be maintained throughout aging by repeat sulfite additions (Ribéreau-Gayon *et al.*, 2000). For this reason, a high limit for total SO_2 (400 mg/l) is permitted in botrytized wines in Europe. A positive side-effect of the high sulfite content is the inhibition of laccase and other oxidase enzymes produced by *Botrytis*. This limits wine browning (Dittrich and Grossmann, 2011). Growth of acetic acid bacteria is also inhibited at this sulfite level.

In Tokaj Aszú, an additional fermentation difficulty arises from the ethanol content already present in the wine at the beginning of fermentation. Thus, a sluggish or early terminated fermentation is more frequent, although it may advance excessively as well. According to the *puttony* number, the minimum levels of residual sugar and extract in Tokaji Aszú are regulated. Termination is typically spontaneous but a combination of moderate sulfiting, filtration, and cooling may be necessary to arrest fermentation at a desired point. Cross-flow microfiltration is occasionally used. Tokaji Aszú generally contains a free SO_2 level lower than German and French versions (20–30 mg/l).

Cessation of fermentation is one of the technical problems in botrytized wine production that needs further research and development. Dimethyldicarbonate (DMDC) is now considered a reliable inhibitor which could replace some of the SO_2. Although DMDC has proven suited for treating wines especially just before bottling, its use in Sauternes production has been investigated (Divol *et al.*, 2005). The results showed that DMDC at a rate of 100–200 mg/l stopped fermentation but did not replace the antioxidant functions of SO_2. Sulfite addition was necessary to limit wine oxidation and yeast reactivation.

Blasi *et al.* (2008) developed an experimental method for removing carbonyl compounds from wine. It used selective liquid–solid extraction, with phenylsulfonylhydrazine as a scavenging agent, bonded to a porous polymer support. The method was efficient for reducing the SO_2 binding power of botrytized wines, without impairing their sensory qualities.

D. Aging and stabilization

Botrytized wines have remarkable aging potential. Most improve with several months to years of in-barrel maturation, followed by many years of in-bottle aging.

German BA and TBA wines are rarely matured in-barrel, since their low alcohol content increases their risk of refermentation. Storage and treatment in tanks under aseptic conditions and an early bottling is typical, although not universally employed.

Premium Sauternes are barrel-aged for 12–18 months and occasionally up to 2 years or more (Ribéreau-Gayon *et al.*, 2000). If the wine was in-barrel fermented, the first racking is performed in December, when the coarsest lees have settled out. Subsequent rackings are performed every 3 months under hygienic conditions. Weekly topping, frequent sulfite additions, and sanitation are necessary to prevent refermentation. Microbial stability, usually determined by plate counts is no guarantee against refermentation. A variety of modern procedures have demonstrated that yeast may remain viable despite not being detected by plate counts (Divol and Lonvaud-Funel, 2005). The VBNC dormancy state is thought to be induced by the presence of SO_2 and the high osmotic potential. Refermentation may occur when free-SO_2 declines.

In Tokaj, at least a 2-year barrel-aging period is compulsory for Aszú wines. Botrytized wines produced similarly, but not barrel matured, can be labeled ''late harvest,'' but not Tokaji Aszú. Aszú wines are intentionally exposed to slow oxidation, although its necessity and duration is debated among producers (Alkonyi, 2000; Kirkland, 1996). The chemical changes associated with barrel aging have not been well studied. Nonetheless, oxidation of alcohols, aldehydes, phenolics, as well as the formation of esters, acetals, and lactones are strongly involved. The longer the aging, the more nuances of dried fruits, chocolate, bread, and coffee develop and add to the primary notes of peach, quince, honey, and botrytis.

Several botrytized wine specialties, other than Aszú, are also matured in the Tokaj cellars. Traditionally, these were exposed to the air for a short period. Today, this is a characteristic only of dry Szamorodni. During this phase a *Saccharomyces* film develops spontaneously on the wine's surface. This donates a flor sherry-like character to dry Szamorodni wines (Alkonyi, 2000; Magyar, 2010). The similarity involves only the flor character, since Tokaji Szamorodni never contains added alcohol. Moreover, its acidity and sugar-free extract content is very high and botrytis notes are present in the taste.

In the older literature this film, which forms on the surface of dry wines only, was misinterpreted as due to the activity of the common cellar mold, *Cladosporium cellare* (syn. *Rhacodium cellare*). It was wrongly associated with the maturation of Aszú wines, as well.

The colonies of this black mold are common on the walls and equipment of Tokaj cellars. *C. cellare* utilizes only volatile compounds which are present in the airspace of the cellar. Since it cannot tolerate ethanol contents above 2% (v/v), it never grows directly on the surface of wine, either sweet or dry. It has no direct impact on the quality of wine, although it beneficially influences the purity and humidity of the air in the cellar (Dittrich, 1977; Magyar, 2006, 2010).

A diversity of maturation concepts and methods is applied to New World botrytized wines, from short, in-tank maturation to the aging in new barriques for several years. Subsequent bottle aging is typical.

Before bottling, the wines normally undergo stabilization, including bentonite fining for protein removal and cold stabilization to avoid tartrate salt crystallization. A unique feature of botrytized wines is the formation and precipitation of calcium mucate crystals, a salt of galactaric or mucic acid (Dittrich and Grossmann, 2011; Würdig, 1976). Their salts are not found in normal wines, and may reach 1–3 mm in long in bottles of old botrytized wines. Because supersaturated solutions remain stable for long periods, cooling is ineffective in donating stability. Reducing the calcium content of wine with DL-tartaric acid addition may reduce the risk for crystalline instability (Würdig and Woller, 1989).

Another unique technological problem in botrytized wines involves the role of *Botrytis* glucans in the clarification. These β-D-glucans, even at concentrations as low as 2–3 mg/l, significantly reduces the filterability of the wine. Concentrations at 50 mg/l make filtration impossible (Wucherpfennig *et al.*, 1984). The breakdown of these glucans is possible with *Trichoderma* glucanases (Dubourdieu *et al.*, 1981; Villettaz, 1990; Villettaz *et al.*, 1984, 1987; Wucherpfennig and Dietrich, 1983).

Botrytized wines need extreme care and sterility during bottling, since the risk of in-bottle refermentation is very marked, in spite of the high sugar content. Hot filling at 50–55 °C would be beneficial in terms of microbiological stability but has not been used because of quality considerations. Fine filtration, including membrane filtration, is widely used, followed by sterile filling and corking. DMDC and potassium sorbate may be added, and frequently are, for microbial stabilization, in addition to sulfur dioxide.

V. HEALTH RELATED ASPECTS OF BOTRYTIZED WINES

A. Health promoting attributes

Botrytized wines have historically been reputed to have extraordinary health benefits. Tokaji Aszú has been presumed to have curative powers for a long time (Kállay *et al.*, 1999). It was actually used as a medicine in the royal courts throughout Europe. Although unique, health promoting constituents have not been identified in botrytized wines, their composition includes a number of physiologically beneficial substances in significantly higher concentrations than found in other wines. The much higher fructose content, relative to glucose, the high organic acid content, and the large amount of minerals have dietary value.

The most significant health-related compounds in wines are polyphenols. It has long been known that grapes and wines contain a large variety of antioxidants, including resveratrol, catechin, epicatechin, and proanthocyanidins (Kállay *et al.*, 1999). In general, they are considered free radical terminators, eliminating reactive oxygen species from the human body. The antioxidant properties and vascular effects of wine phenolics, and their impacts on lipid metabolism and life span have been extensively studied, as reviewed by Dávalos and Lasunción (2009) and Bertelli (2009). Resveratrol and its derivatives, as well as proanthocyanidins, play a crucial role in the cardioprotective properties of grapes and wines.

Due to the long maceration on skin, red wines are particularly rich in phenolic compounds, having higher antioxidant capacity and also higher resveratrol content than white wines. Of the high number of studies on this field, only a few focused on the botrytized white wines.

In comparison with normal white wines, botrytized wines generally contain much higher quantities of polyphenols (Poor Nickfardjam *et al.*, 2002) elevating their antioxidant capacity. Measuring polyphenol content, as well as antioxidant capacity as TEAC (trolox equivalent antioxidant capacity) value (Re *et al.*, 1999) in German and Tokaj botrytized wines, Pour Nikfardjam *et al.* (2006) reported much higher values (Table 6.8) than found in nonbotrytized wines (Pour Nikfardjam *et al.*, 1999). This probably results due to the concentrating effect of grape dehydration, and berry maceration on the skins before pressing. Due to the much longer maceration time, and the alcoholic environment during maceration, Tokaji Aszú has higher values for both parameters, sometimes comparable with those found in red wines (Kállay *et al.*, 1999; Pour Nikfardjam *et al.*, 2006).

Among polyphenols, resveratrol and its derivatives are thought to play a special role. Resveratrol occurs naturally in grapes in both *cis*- and *trans*-isomers, and in their respective glucosides (*cis*- and *trans*-piceids). All forms inhibit the oxidation of low density lipoprotein (LDL) and additional benefits. Resveratrol occurs in most red wines but is undetectable or occurs in negligible amounts in dry white wines (Pour Nikfardjam, 2002).

Since it is produced in response to fungal attack (see Jeandet *et al.*, 2002), its elevated level in botrytized grapes and wines might be expected. However, studies with Tokaji Aszú (Kállay, 2005) revealed only low quantities of resveratrol isomers (0–2.39 mg/l). In contrast, their

TABLE 6.8 Resveratrol, piceid, total phenols, and antioxidant capacity (TEAC) of some Tokaji and German botrytized wines. Mean values are in parentheses (adapted from Pour Nikfardjam *et al.*, 2006, and with permission from Elsevier)

	n[a]	*trans*-Piceid (mg/l)	*cis*-Piceid (mg/l)	*trans*-Resverat-rol (mg/l)	*cis*-Resvera-trol (mg/l)	Total piceid + resveratrol mean (mg/l)	Total phenols (mg/l)	TEAC (Mmol/l)
Tokaji Szamorodni	3	0[b]	0	0	0–2.8 (0.93)	0.93	537–787 (685)	3.2–4.9 (4.27)
Tokaji Aszú 5–6 puttonyos	11	0–1.8 (0.75)	0–6.6 (1.99)	0–0.4 (0.04)	0–2.5 (0.43)	3.23	621–1403 (846)	2.0–7.4 (5.42)
Auslese	5	0–3.4 (0.68)	0–2.9 (0.58)	0–0.4 (0.14)	0 (0.0)	0.14	248–615 (350)	0.7–1.8 (1.34)
Beerenaulese	4	0–1.5 (0.50)	0–0.7 (0.30)	0–0.5 (0.20)	0–0.6 (0.15)	0.35	377–498 (422)	0.6–1.6 (1.13)
Trockenberen-auslese	3	0–0.5 (0.17)	0–1.2 (0.40)	0 (0.0)	0 (0.0)	0.0	479–747 (609)	2.4–1.4 (1.87)

[a] Number of wine samples.
[b] 0, not dectectable, <0.3 mg/l.

occurrence was somewhat higher in dry white wines. Pour Nikfardjam *et al.* (1999, 2006) demonstrated that the resveratrol and piceid contents of Tokaj Aszú (2.5 mg/l) was higher than found in German botrytized wines (0.9 mg/l). This most likely relates to the long skin-contact time for Aszú wines (Table 6.8). The low resveratrol concentrations of botrytized wines can be explained by the high activity of stilbene-oxidase in *Botrytis*. This laccase oxidizes resveratrol and piceid to inactive ingredients (Jeandet *et al.*, 1995).

Landrault *et al.* (2002) also demonstrated that during noble-rot development in Sauvignon or Sémillon grapes, levels of *trans*-astringin, *trans*-resveratrol, *trans*-piceid, and pallidol are low (<0.5 mg/kg for grapes). Only the oligomer, viniferin, was detected in relatively high concentrations (2 mg/kg), reaching a maximum early during infection. A flavonol, astilbin (having hepatoprotective effects), reached as high as 30 mg/kg in Sauvignon grapes. In botrytized Sémillon wines, they found 0.08–0.17 mg/l viniferin, comparable with the concentrations found in red wines.

B. Biogenic amines

Biogenic amines in wine and fermented foods are formed primarily via the microbial decarboxylation of amino acids. Examples, such as histamine, tyramine, and phenylethylamine are toxic, especially in alcoholic beverages. Ethanol can inhibit the monoamino oxidase responsible for amine detoxification (Maynard and Schenker, 1996). Histamine can induce allergenic reactions in humans, such as rashes, edema, headaches, hypotension. Tyramine and phenylethylamine can cause hypertension and other symptoms related to the release of noradrenaline.

Numerous research studies have been published dealing with the level and formation of biogenic amines in wine (see Moreno-Arribas and Polo, 2009), but only a few relate to *Botrytis*-affected grapes or wines (Eder *et al.*, 2002a; Hajós *et al.*, 2000; Kállay, 2003; Kiss *et al.*, 2006; Sass-Kiss and Hajós, 2005; Sass-Kiss *et al.*, 2008).

Malolactic fermentation is often viewed as the main source of polyamines in wine production (Marcobal *et al.*, 2006). Thus, their presence is more significant in red wines than in white ones. Of white wines, sparkling wines, biologically aged wines, and botrytized wines might be the most susceptible to biogenic amine formation.

Eder *et al.* (2002a) investigated numerous must and wine samples made from healthy and *Botrytis*-infected grapes. Total amines were significantly higher in infected grape material, for both white and red cultivars. Higher values were mainly due to the presence of isopentylamine and phenylethylamine (Table 6.9). The contents of other biogenic amines increased only slightly. Histamine contents generally were low

TABLE 6.9 Occurrence of some biogenic amines in botrytized wines, in comparison with nonbotrytized wines

	n	Histamine	Tyramine	Putrescine	Phenyl-ethylamine	3-Methyl butylamine	Reference
Nonbotrytized wines, Austria	15	0–2.9 (1.13)	0–1.54 (0.38)	1.1–4.2 (2.36)	0–1.4 (0.41)	0–6.95 (3.67)	Eder *et al.* (2002a,b)
Austrian botrytized wines	16	0–5.9 (1.81)	0–4.1 (0.80)	0.6–4.9 (2.69)	1.5–14.9 (5.59)	2.5–41.8 (14.53)	Eder *et al.* (2002a,b)
Nonbotrytized Wines, Hungary	17	n.d.–5.5 (1.7)	0.5–7.8 (3.7)	1.8–16.6 (8.3)	n.d.–15.5 (5.7)	–	Kállay and Sárdy (2003)
Tokaji Aszú	21	n.d.–0.1 (0.07)	0.7–2.9 (1.78)	1.6–3.6 (2.28)	9.6–19.1 (14.74)	15.7–23.9 (19.19)	Sass-Kiss *et al.* (2008)
Botrytized wines other than Tokaji[a]	24	n.d.–15.4 (2.14)	n.d.–10.0 (1.79)	0.3–14.4 (4.30)	0.04–20.2 (3.52)	0.06–22.2 (5.26)	Sass-Kiss *et al.* (2008)

Minimum and maximum values; mean values in parentheses (mg/l).0, not detected, $<$0.25 mg/l for Austrian wines, and $<$0.1 mg/l for the other wines.

[a] Botrytized wines from Austria (4), Spain (4), France (3), Italy, Germany, Portugal, Slovakia, and the USA.

(maximum values of 5.89 mg/l). Analyses of 22 Prädikat wines showed total amine contents related to the sugar concentration of must (roughly equivalent to the degree of botrytization). Investigations on the polyamine contents of *aszú* grapes in Tokaj revealed similar results. The total polyamine content of Aszú grapes was considerably higher, and the amine composition significantly different from those of intact grapes (Kiss *et al.*, 2006). The authors confirmed increases in 3-methyl-butylamine (isopentylamine) and phenylethylamine contents in botrytized grapes, as well as higher concentrations of *i*-butilamine, agmantine, and spermidine. In contrast, histamine concentration decreased in association with fungal infection. The relatively high spermidine content was metabolized during vinification (to 0–5 mg/l), whereas the phenylethylamine concentration rose (from 8 to 18 mg/l). Histamine and cadaverine contents remained low (Hajós *et al.*, 2000).

In a recent study, Sass-Kiss *et al.* (2008) found the biogenic amine content higher in Tokaji Aszú than in other botrytized wines. High concentrations of 3-methyl-butylamine, 2-methyl-butylamine were found in all botrytized wines, but the concentrations were considerably higher in Tokaji Aszú (Table 6.9). Of the compounds studied (biogenic amines, organic acids, and mineral elements), the authors suggest biogenic amines are most suitable components for authenticating the origin of Tokaji Aszú. Another characteristics of Tokaji Aszú is its high (>100 mg/l) serotonin content (Kállay, 2003, 2005). This biogenic amine, not investigated by the other authors, is an important neurotransmitter and occasionally used in treating certain depressions (Kállay, 2005). Its concentration is negligible in most white and red wines.

Although the polyamine content of botrytized wines are significantly higher than those in other white wines, these concentrations, particularly those of the critical histamine and tyramine, fall below the values typical of red wines (Marcobal *et al.*, 2006). Considering the occasional and moderate consumption of botrytized wines, their amine content is not a health issue.

C. Mycotoxins

Foods and beverages exposed to fungal activity should be investigated relative to mycotoxin presence. Of those known, only ochratoxin-A (OTA) seems relevant to wine production (Hocking *et al.*, 2007). OTA is mainly produced as a secondary metabolite by some *Aspergillus* and *Penicillium* species. OTA is considered a potential human carcinogen. It is also nephrotoxic, hepatotoxic, teratogenic, and immunotoxic in several animals. In humans, it is believed to accumulate in body tissue, although its effects have not been completely clarified (Ringot *et al.*, 2006).

Occurrence of OTA in wines was first reported by Zimmerli and Dick (1996), generating extensive research activity into its origin and control (see Malfeito-Ferreira *et al.*, 2009; Stratakou and van der Fels-Klerx, 2010). The occurrence of OTA is higher in southern Europe than in northern countries, and higher in red and certain sweet wines than in white wines (Stratakou and van der Fels-Klerx, 2011). Although the toxin was named after *Aspergillus ochraceus*, *Aspergillus carbonarius* and other black aspergilli (*Aspergillus niger*, *Aspergillus tubilensis*, *Aspergillus brazilensis*) are the species most responsible for toxin production on grapes in warmer climates. In colder climates, *Penicillium* species have been found principally responsible for OTA contamination in several agricultural products, including cereals (Pitt, 2000). This also appears to apply to grapes as well (Torelli *et al.*, 2005; Varga *et al.*, 2007). On the basis of numerous surveys, the European Commission has established 2 μg/kg as the maximum allowable level for OTA in wine and grape products, excluding fortified wines (Commission Regulation, 2006).

Botrytized wines, being produced from mold-affected grapes, might be expected to be contaminated with OTA. Although *Botrytis* itself has never been reported to produce mycotoxins, associated *Penicillium* and *Aspergillus* species might be involved in toxin production. To date, studies on botrytized wine have either not detected or found low OTA levels.

OTA levels in Aszú wine fell from 0.53 μg/l after maceration to close to the detection level (0.02 μg/l) during fermentation (Kállay, 2005). In addition, Kállay and Bene (2003) detected very low concentrations (0.024–0.193 μg/l), and only in 4 of 10 different Aszú wines.

Eder *et al.* (2002a,b) surveyed 117 Austrian wines including 55 potentially botrytized Prädikat wines (Auslese, BA, Ausbruch, TBA) for OTA. None of the samples contained the toxin at a detectable levels. In 121 different wines studied by Valero *et al.* (2008), the wines with the highest OTA contents were those produced from must fortified before fermentation (4.48 μg/l) and those made from sun-dried grapes (2.77 μg/l). Wines affected by noble rot contained no detectable OTA. Icewines and late-harvest wines were also not contaminated. Nonetheless, an elevated OTA concentration has been reported in some South African botrytized wines (Stander and Steyn, 2002).

VI. SUMMARY AND CONCLUSIONS

Natural sweet wines produced from *Botrytis*-affected grapes are among the highest quality and most expensive wines in the world. From three traditional centers, Tokaj, Rheingau, and Sauternes, the production of botrytized wine has spread throughout the world, including northern Italy, Australia, New Zealand, South Africa, and California. The main differences among these various wines involve the level of botrytization, berry dehydration, the ratio of botrytized to uninfected grapes, the amount of sulfur dioxide added, and the aging procedure (from reductive to slightly oxidative).

The production of botrytized wines is initially limited by the special conditions required for noble rot development. Noble rot develops concurrent with berry dehydration. In the process, compounds present in the overripe grapes and produced by *B. cinerea* become extremely concentrated. The result is the generation of wines with very high sugar and extract contents, and exceptional aroma richness.

In the second half of the twentieth century, the mechanism of fungal infection, as well as the basic physical and biochemical changes during berry ripening, has been extensively studied. Among the most important changes are increases in sugar and acidity, as well as accumulation of glycerol, gluconic, galacturonic, galactaric acids, and special polysaccharides (β-glucans), and a unique sensory aroma derived from *Botrytis*.

The nature of the *Botrytis* aroma compounds has been subjected to extensive research. In addition to the older findings about the importance of hydroxy-, oxo-, and dicarboxylic acid esters, acetals, and some special γ- and δ-lactones, the role of volatile thiols has recently been elucidated. Nonetheless, additional research is needed to identify odor active compounds that are specific for botrytized wines.

Due to the concentrating effect of noble rot as well as the maceration widely used before pressing, these wines are rich in polyphenols. They provide more antioxidants than other white wines. This particularly applies to Tokaji Aszú, where maceration occurs in an alcohol medium (fermenting must or young wine). However, the concentration of resveratrol and its derivatives is not significantly higher than in normal white wines, due to their decomposition by *Botrytis* oxidases.

In accordance with the enhanced microbial activity on the surface of botrytized grapes, botrytized wines contain more biogenic amines than normal wines. However, these values do not exceed those measured in red wines. Despite contamination of the infected berries by saprophytic fungi, the mycotoxin content of these wines is low—with only OTA being occasionally detected. At the amounts present, it is not a health concern.

Future research needs to study the chemical, biological, or immunoactive nature of provenance authentication. In addition, methods for the objective quality assessment of noble-rotted berries are required. Better control of alcohol fermentation (including predictable dynamics and reduction of volatile acid) through the use of mixed cultures of *S. cerevisiae*, *S. uvarum* and/or non-*Saccharomyces* species would be beneficial. Improved means scheduling the terminating alcohol fermentation, as well as advances in stabilization and clarification methods (with special regards to fungal colloids) are also among the most urgent targets of further research.

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