Botrytized Wines: A Review

N.S. Thakur¹, Abhimanyu Thakur¹*, V.K. Joshi² and Satish K. Sharma³

¹Department of Food Science and Technology, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan, HP 173230, India
²Former, Professor and Head, Department of Food Science and Technology, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan, HP, India
³Joint Director Research (Horticulture), Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan, HP, India

*Corresponding author: abhimanyuthakurprashar@gmail.com

Abstract

Botrytized wines are the sweet wines made from grapes that have been botrytized with the fungus Botrytis cinerea Pers. (B. cinerea). This fungus grows on grapes and are responsible for causing Botrytis bunch rot and/or grey mould rot in many vineyards around the world. Under specific climatic conditions fungus produces pourriture noble or noble rot which is a desirable stage for the production of botrytized wines from these infected berries. The infection has been found to cause dehydration of the grape berries along with other significant modifications like skin-cell degradation, glucose degradation and generation of glycerol and aromatic compounds. During rotting aromatic compounds have been found to be highly modified and lead to the formation of characteristic compounds like 3-hydroxy-4,5-dimethyl-2-furanone, furfural, benzaldehyde, phenylacetaldehyde and benzyl cyanide which are known for their distinctive aroma. To exploit the higher concentration of various chemical constituents including sugars the sweet white table wines are produced commercially from these infected berries. The botrytized wines are rich source of polyphenolic antioxidants which provide various health benefits to the consumers.

Keywords: Botrytis cinerea, botrytized wines, noble rot, fungi, fermentation

Wine is one of the oldest known beverage to mankind with a wonderful diversity in styles and quality. It is produced by complete or partial fermentation of fresh grapes, crushed or not, or of grape must (Ribereau-Gayon et al. 1998). Botrytized wines are natural sweet wines produced from the withered and shrivelled berries infected with B. cinerea (Magyar and Soos, 2016). However, this fungus is also held responsible for bunch rot or grey mould rot but under certain climatic conditions, noble rot is produced which is of economic importance in the production of botrytized wines. Moist nights, foggy mornings and dry days during the infection of B. cinerea are responsible for shrivelling of berries due to loss of moisture which further leads to noble rot stage (Ribereau-Gayon et al. 1980). On the other side, the unfavourable conditions like heavy rainfall and high humidity have been found to favour the bunch rot which has a negative impact on wine production as it affects the fermentation process and sensory properties of wine (Hornsey, 2007; Morales-Valle et al. 2011; Gubler et al. 2013). The initial attack of the fungus is near the cell wall and it triggers a biochemical attack on the plant tissue and cells to aid the spread of infection. During fungal infection, various hydrolytic and oxidative enzymes are produced which transform many components of the grape tissues and the juice (Magyar, 2006). The infected berries are brown in colour and further dried into a sort of moist raisin with low acid, low nitrogen and reach extremely high levels of sugars content.
nearly to 30-40%. In this process, there is an increase in sugar alcohols like glycerol, arabinitol, mannitol, sorbitol and inositol whereas glucose to fructose ratio and tartaric acid concentration of the infected berries decreases. The higher ratios of glycerol to gluconic acid indicate the growth of true noble rot, whereas, the lower ratios suggest sour rot (Ribereau-Gayon, 1988). Polysaccharides are decomposed by the fungal enzymes leading to the accumulation of β-glucans, polyols (mannitol, erythritol and meso-inositol), arabinose, rhamnose, mannose, galactose, xylose and galacturonic acid (Jackson, 2008). These polysaccharides especially β-D-glucan may cause clarification problems in wine making process (Dubourdieu, 1982; Villettaz et al. 1984).

*B. cinerea* affects the composition of fruit acids during noble rotting process and total acidity of grapes decreases during the infection process. Various nitrogenous and phenolic compounds like amino acids, ammonium salts, total phenol content, total tannin and anthocyanins are significantly affected. During rotting, aromatic compounds are highly modified and new aromatic compounds are formed with noble rot which imparts a honey like or roasted aroma to the wine (Shimizu et al. 1982). Comparison of some parameters of juice from healthy and *B. cinerea* infected grapes has been made in Table 1. Mould and associated acetic acid bacteria consume some portion of the grape sugars, but it is countered by an increase in sugar due to dehydration of berries. The increase in the sugars content in the infected berries has led to the commercial use of *B. cinerea* in the production of sweet white table wines. Various aspects like types of botrytized wines, *B. cinerea* pathogenesis, changes in berry or juice composition and estimation of *B. cinerea* during infection process with respect to the production of botrytized wines have been reviewed in this paper.

**History of botrytized wines**

The production of botrytized wines for the first time has been reported in Tokaj region of Hungary (ca. 1560) and Schloss Johannisberg region of Germany (ca 1750). While in France, the production of botrytized wines had been started in Sauternes region between 1830 and 1850 (Jackson, 2008). *Tokaji Aszu* was the first intentionally made wine from botrytized grapes (Magyar, 2011). In 1775, owner of Schloss Johannisberg forgot to send permission to start the harvesting and the advantage of harvesting grapes very late in the Rheingau region in Germany was originated accidentally (Thudichum and Drupe, 1872). For the production of botrytized wines, various pickings of botrytized fruits in France have been explained during 1863 and procedure of wine making from botrytized grapes was described as early as 1872 (Shaw, 1863; Thudichum and Drupe, 1872). *Botrytis* infection of Semillon grapes of Crimea in Russia was reported in 1899 which further led to the production of a sweet table wine in the same year (Khovrenko, 1910). The sweet white wines of Gramdjo in northern Portugal are also produced from the *B. cinerea* infected grapes (Everett, 1954). Nelson and Nightingale (1959) carried out the first commercial implementation of *B. cinerea* inoculation. They inoculated palletized trays of grapes with *B. cinerea* produced in 1 ft² custom-made petri dishes containing a 2 per cent agar-grape juice medium. During later period, several workers conducted investigations to produce botrytized wines by adding *Botrytis*, or enzymes from *Botrytis*, directly to juice or must and had succeeded in introducing sensory characteristics of botrytized wines (DeSoto et al. 1966; DeJong et al. 1968; King et al. 1969; Akau et al. 2004). Botrytized wines are also produced to a limited extent in Australia, New Zealand, South Africa, United States and all the regions of Europe where favourable conditions for noble rot persists (Jackson, 2008).

**TYPES OF BOTRYTIZED WINES**

**Tokaji Aszu**

*Tokaji Aszu* was the first botrytized wine prepared from the grape berries infected with *botrytis* as reported by Jackson (2008) in Tokaj, Hungary. These *aszu* wines are prepared by mixing young white wines, or juice derived from the healthy grapes, with paste made from pulverized *aszu* berries (Jackson, 1994; Karagiannis, 2011). A very small amount (1 to
1.5 litres) of Aszu Eszencia is collected from 30 liters of juice extracted from botrytized berries. The collected Eszencia is kept in small wooden barrels for further fermentation and maturation. The categories of Aszu are based on amount of Aszu added to the mixture and termed as puttony (a puttony equals to 28-30 liters). Aszu wines are matured for a time period of 3 years and out of this time interval, use of small oak barrels for 2 years is compulsory (Magyar, 2011). The alcohol content in the prepared wine varies from 12 to 14 % (Jackson, 1994). The chemical composition of Tokaji wines has been depicted in Fig. 1 (a and b). Fordias, Malslas and Szamorodni are well known Tokaj specialty wines internationally besides Eszencia and Aszu (Kirkland, 1996 and Eperjesi, 2010).

**German Botrytized wines**

In Germany, the production of botrytized wines from noble rot grapes had started in 1775 at Schloss Johannisberg vineyards (Robinson, 2006). Rulander, Riesling, Gewurztraminer, Silvaner, Scheurebe and Huxelrebe are the predominant cultivars grown, which are prone to noble rot (Magyar, 2011). These wines are divided into three various categories namely Auslesen wines (may or may not be Botrytis concentrated), Beerenauslesen wines and Trockenbeerenauslesen wines. Out of these three Beerenauslesen and Trockenbeerenauslesen wines are made from B. cinerea attacked berries which possess higher sugars content. These wines have relatively

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**Table 1: Comparison of juice from healthy and B. cinerea infected grapes**

<table>
<thead>
<tr>
<th>Component</th>
<th>Sauvignon berries</th>
<th>Semillon berries</th>
<th>Germany</th>
<th>Tokaj</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy</td>
<td>Infected</td>
<td>Healthy</td>
<td>Infected</td>
</tr>
<tr>
<td>Fresh weight/100 berries (g)</td>
<td>225</td>
<td>112</td>
<td>202</td>
<td>98</td>
</tr>
<tr>
<td>Sugar content (g/liter)</td>
<td>281</td>
<td>326</td>
<td>247</td>
<td>317</td>
</tr>
<tr>
<td>Acidity (g/litre)</td>
<td>5.4</td>
<td>5.5</td>
<td>6.0</td>
<td>5.5</td>
</tr>
<tr>
<td>Tartaric acid (g/litre)</td>
<td>5.2</td>
<td>1.9</td>
<td>5.3</td>
<td>2.5</td>
</tr>
<tr>
<td>Malic acid (g/litre)</td>
<td>4.9</td>
<td>7.4</td>
<td>5.4</td>
<td>7.8</td>
</tr>
<tr>
<td>Citric acid (g/litre)</td>
<td>0.3</td>
<td>0.5</td>
<td>0.26</td>
<td>0.34</td>
</tr>
<tr>
<td>Gluconic acid (g/litre)</td>
<td>49</td>
<td>7</td>
<td>165</td>
<td>25</td>
</tr>
<tr>
<td>pH</td>
<td>3.4</td>
<td>3.5</td>
<td>3.3</td>
<td>3.6</td>
</tr>
</tbody>
</table>

higher sugars and lower alcohol content as compared to Tokaji wines (Fig. 2a and 2b).

**French Botrytized wines**

French botrytized wines have been found to contain higher alcohol content (≥ 11-13%) with high sugar concentration than the German wines (Jackson, 2008). French botrytized wines are white and sweet which are produced in Sauternes which is located near two rivers namely Garonne and Ciron, where favourable conditions prevail for the development of noble rot. The harvesting is carried out once and the separated botrytized or infected grapes are used for the production of dry wines (Karagiannis, 2011). The intentional production of botrytized wines from infected grapes had started two centuries later in France than in Tokaj (Jonson and Robinson, 2001).

**Pathogenesis or botrytization process**

Pathogenesis process, role of fungal enzymes in tissue invasion, pre-disposing factors for berries infection, artificial inoculation and *in vitro* establishment of *B. cinerea* are some of the important aspects for the production of botrytized wines.

**Fungal infection and life cycle**

*B. cinerea* is an Ascomycete fungus, belonging to order Halotiales and family Sclerotiniaceae. The dissemination of fungus takes place through resistant forms like sclerotia and conidia which are dispersed by wind and rain water, and leads to infection. Two types of infection usually occur on grape berries. First is the grey rot which occurs due to consistently wet or humid conditions and other is the noble rot which occurs when wet conditions are followed by dry conditions. Grape berry moth larva (*Lobesia botrana*) is one of the important vectors in dissemination of fungus by carrying the conidia both on and in their bodies (Fermaud and Le Menn, 1989). Under moist conditions the conidia germinate on the host surface producing a germ tube which further develops into appressorium and penetrates the host surface. Due to weak enzymatic profile of the fungus, it can’t penetrate directly and requires physical injuries/wounds or natural openings for inserting penetration peg into the host tissue (Rijkenberg *et al.* 1980). After penetration, the underlying cells are killed and the fungus establishes a primary lesion in which necrosis and host defense response may occur (Prusky, 1996 and Jan, 2005). The infection cycle is completed in 3-4 days depending on the type of host tissue attacked. The attachment of conidia to the host tissue is a two step process. First step involves weak adhesive forces resulting from hydrophobic interactions between host and conidial surface and after several hours of inoculation the conidia gets germinated and leading to stronger binding (Doss *et al.* 1993; Doss *et al.* 1995). High humid conditions (≥ 93 %) have been reported to be essential for conidia germination and penetration.

**Fig. 2 (a and b):** Chemical composition of *Beerenauslesen* (B1, B2, B3) and *Trockenbeerenauslesen* (T1, T2) wines (Based on Watanabe and Shimazu, 1976)
of host epidermis (Williamson et al. 1995). After attachment and germination of conidia the host tissue invasion takes place by means of active penetration or passive ingress. It has been found that after physical penetration if the fungus was not capable of growing hyphal branches, it further attacked on the berries by releasing various chemicals or enzymes.

Changes produced by fungi

During invasion process the fungus uses two chemical weapons at this point: high-molecular-weight enzymes that break down the cell wall and membrane, and leads to tissue maceration, and production of low-molecular-weight toxins that kill the plant cells as the hyphae advances through the host tissue. As the conidial germination starts, the endopolygalacturonase enzyme is synthesized (Verhoeff and Warren, 1972). The pectin compounds are degraded completely and accompanied by alteration of cell wall composition greatly by other enzymes like exopolygalacturonase, pectin methylestrase, transeliminase, phospholipase and protease (Shepard and Pitt, 1976; Movahedi and Heale, 1990). After the complete mycelium invasion into pectocellulosic cell walls, the white grape berries convert into a characteristic chocolate hue known as “pourii plein”. The filaments produced on mycelium then have been found to pierce through the cuticles or taking a route through the various fissures already used for penetration. After invasion process small, circular, faintly cleared spots are the first symptoms visible on berries. The berries become dark colored with typical greyish hairy mycelium all over their surface but under high humid conditions the mycelial growth may be cottony and white. Often the fungus can be seen growing along the cracks or splits on the berries. After differentiation, conidiophores are developed on these filaments, which are erect, hyaline, unbranched or seldom branched. The fungus infected cell walls of berries have been found to be so greatly modified that they are no longer functional. The hydration of the berry cells however varies according to climatic conditions and usually leads to a characteristic withering which has been found to ultimately cause the cytoplasmic death of the epidermal cells. This stage is known as confit or “pourri roti”. The fungal attack leads to the secretion of phytoalexins which are stilbenic derivatives with fungicidal properties. Stilbenes oligomers (viniferin and pallidol) are having anti-cancerous property and play an important role in protecting lipoproteins from oxidative damage (Landrault et al. 2002). The infected berries are brown in colour and dry into a sort of moist raisin with low acid and low nitrogen content, and are expected to reach extremely high levels of sugars content (30-40%). These berries are therefore, further picked up and utilized for the preparation of highly aromatic sweet wines.

Favourable conditions for noble rot

Grey mould is one of the serious diseases in the vineyards but when the infection occurs at favourable environmental conditions it plays an important role in the production of botrytized wines. Favourable conditions for noble rot development in grapes have been determined and are summarised in Table 2.

Table 2: Favourable conditions for noble rot development in grapes

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Optimum for noble rot development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maturity of berries</td>
<td>Fully ripened</td>
</tr>
<tr>
<td>Intact berries</td>
<td>Avoid attack of other non-desirable pathogens</td>
</tr>
<tr>
<td>Water supply</td>
<td>In excessive</td>
</tr>
<tr>
<td>Temperature</td>
<td>15-20 °C</td>
</tr>
<tr>
<td>Humidity</td>
<td>90% or above, drizzle, dew, morning mist</td>
</tr>
<tr>
<td>Hot sunny days</td>
<td>Good for conidial dispersal</td>
</tr>
<tr>
<td>High evaporation rate</td>
<td>Juice concentration</td>
</tr>
<tr>
<td>High wind velocity</td>
<td>Conidial dispersal</td>
</tr>
<tr>
<td>Nature of clone</td>
<td>Number of stomata Skin thickness Anti-fungal substances</td>
</tr>
</tbody>
</table>

Source: Thakur and Sharma, 2011.

Bulit and Lafon (1970) have reported the mean temperature range of 15-20 °C with 90 per cent relative humidity as the most favourable conditions for the development of fungus. Conidia can however
germinate within a wide range of temperature (10-25 °C) though the optimum temperature for its germination is 18 °C (Ribereau-Gayon et al. 2000). The berries are prone to bursting at high temperature, whereas, high evaporation rates are responsible for the production of concentrated juice (Nelson, 1958). As the B. cinerea attack is less in hot and dry conditions, artificial inoculation of berries with fungus is also practiced. For artificial inoculation of mature grapes with B. cinerea, the berries are kept at a temperature range of 20-25 °C along with >90 per cent relative humidity for 24 h (Nelson et al. 1963). The berries are exposed to cold and dry conditions (20 °C and 50 % RH) to inhibit the fungal growth and facilitate the dehydration of berries.

Artificial inoculation of fungus

The berries produced through artificial inoculation of fungus are high in sugar and low in acid content with similar aromatic profile of naturally botrytized grapes (Nelson and Amerine, 1956; Nelson and Nightingale, 1959). Wang et al. (2017) have sprayed grapes clusters with ground hyphal powder of B. cinerea dissolved in 20 g/l glucose with spore concentration of 1 × 10⁶ CFU/ml and further reported that botrytized wines were rich in aroma compounds than dry white wine and delayed harvest sweet wines. Other compounds like esters, fatty acids, lactones, furans, phenolic acid derivatives, thiols and volatile phenols were found to be increased in noble rot wines, and ethyl isovalerate, 2-methylsulfanyl-hexanol, ethyl 4-hydroxybenzoate and 2-nonanone were the specific aroma components in noble rot wines. During inoculation of these berries, the favourable conditions of temperature (20-25 °C) and humidity (> 90 % for 24 h) were maintained for the fungal growth.

Many attempts have been made for the in vitro establishment of B. cinerea that is production of botrytized wines with the direct addition of inoculum into the juice or must rather than on the grape berries (DeSoto et al. 1966; DeJong et al. 1968; King et al. 1969; Nelson and Amerine, 1957; Watanabe and Shimazu, 1978; Akau et al. 2004). Akau et al. (2004) have reported that B. cinerea can be produced in liquid as well as solid-phase culture in different media and the solid state fermentation process can be used as a large scale production method for controlled introduction of B. cinerea into the vineyards as the solid state fermentation products could be stored longer than liquid fermentation products. The fermentors have been developed for the treatment of grape must with this fungus and the addition of compound like cyclic adenosine mono phosphate into the fermentors can help in the controlled production of gluconic acid and glycerol during the fermentation process. But it is difficult to produce special aroma and to control the levels of acetic acid and acetaldehyde in this process. So the commercial production of these wines is a costly affair due to these complexities.

Chemical changes during botrytization

Dehydration of the berries due to B. cinerea infection has been reported to produce physico-chemical changes which are of prime importance in botrytized wine production. These physico-chemical changes and their effects on the quality of botrytized wine produced have been reported by various researchers (Table 1).

Sugars and polysaccharides

Shriveling of the berries leads to the concentration effect on sugars which are further utilized for the production of botrytized wines. The initial sugar content of berries may rise as high as 30-40 per cent but the cost of production of fruit is high as there is reduction of grape volume (Dubos, 1999). In Sauternes and Tokaj, the sugars content of botrytized berries have been found to reach upto 350-700 g/l (Magyar, 2011; Kerenyi, 2013; Magyar and Soos, 2016). Tosi et al. (2013) carried out induced botrytization with different strains of B. cinerea (Bc27, Bc46 and Bc52) and observed an increase in residual sugars from 73.60-77.80 g/l (control) to 107.40-146.40 g/l (botrytized) in Recioto di Soave wines. It is reported that the conidia and young mycelium of fungus contained the enzymes similar to TCA cycle, Embden-Meyerhof pathway, the monophosphate shunt and glyoxylate cycle (Doneche, 1989). These
enzymes are found to be responsible for glucose catabolism and further oxidation of glucose is similar to the Entner-Doudoroff pathway (EDP) and cellular material is synthesized during the exponential growth phase. The glucose is oxidized directly by glucose oxidase leading to accumulation of gluconic acid during stationary phase. The accumulation of glycerol and gluconic acid are the chief indicators of B. cinerea infection. The gluconic acid level in the wine prepared from botrytized fruits have been found to be ranged from 1 to 5 g/L whereas it is 0.5 g/L in the wine prepared from clean fruits (Ribereau-Gayon, 1988). The glycerol concentration was observed to be the highest during the pourri plein stage and is about 50-60 micromoles per berry, whereas, it is 10-40 micromoles per berry at the “pourri roti” stage along with 5-7 g/L of glycerol in the must. During intense shriveling and dehydration, the glycerol content may exceed 30 g/l (Dittrich and Grossmann, 2011). Lorenzini et al. (2013) have studied the composition of juice obtained from grapes inoculated with B. cinerea and reported an increase in glycerol (0.03 to 2.61 g/L) and gluconic acid content (0.02 to 0.13 g/L) with respect to the juice from non-inoculated berries. In addition to glucose, other polysaccharides have been found to decomposed by the fungal enzymes leading to the accumulation of polyols (mannitol, erythritol and meso-inositol), arabinose, rhamnose, mannose, galactose, xylose and galacturonic acid (Bertrand et al. 1976). These polysaccharides especially β-D-glucan may cause clarification problems in wine and even a little concentration (2-3 mg/l) can retard filtration process (Villetaz et al. 1984). As these glucans are located just under the skin, so the berries are harvested gently along with slow pressing so as to minimize their release into the juice (Jackson, 2008). The enzymes like β-glucanase hydrolyze the β-glucan and can improve the filtrability of wines prepared from botrytized grapes.

Acids

Tartaric and malic acid are the predominating acids present in the grapes berries, whereas, other acids like citric, isocitric, aconitic, glutaric, fumaric, shikimic, lactic and succinic acids are found in minute concentrations. B. cinerea is known to affect the composition of these fruit acids during noble rotting process. Malic acid degradation is as high as 70 to 90 per cent, whereas, tartaric acid is degraded up to the extent of 50 to 70 per cent (Doneche, 1990). Lorenzini et al. (2013) studied the composition of juice obtained from grapes inoculated with B. cinerea and reported a lower tartaric acid content (1.99 g/L) with respect to juice from non-inoculated berries (3.48 g/L). Lactic and succinic acids were formed during alcoholic and malolactic fermentation and the average concentration of succinic acid in wine was found to reach 1 g/L (Ribereau-Gayon et al. 2000). Tosi et al. (2013) carried out induced botrytization with different strains of B. cinerea (Bc27, Bc46 and Bc52) and observed an increase in acetic acid content from 0.37-1.00 g/l (control) to 0.61-1.41 g/l (botrytized) in Recioto di Soave wines.

Nitrogen and amino acids

During infection process, there is a detrimental effect on assimilable nitrogen and various nitrogen containing components like ammonium salts, amino acids and peptides, and nucleic acid derivatives. There is 30-80 per cent degradation of total amino acid content in infected grapes (Rapp and Reuther, 1971; Dittrich and Sponholz, 1975). The exocellular proteolytic enzymes like proteases and amino oxidases act upon the proteins and consequently, the nitrogen component of amino acids is liberated. The fungus utilizes this liberated nitrogen for various metabolic processes during infection and ammonia is secreted at the end of fungal growth. B. cinerea is responsible for the degradation of ammonia nitrogen and vitamins like thiamin and pyridoxine. The effect of B. cinerea on nitrogenous compounds has been shown in Fig. 3 and 4.

Phenolic compounds

The phenolic composition of grape skin has been found to be significantly affected by B. cinerea infection. A significant decrease in TPC (total phenolic content) was observed in seeds from healthy to infected grapes (51.1 vs 42.6 mg GAE/g dw, respectively), whereas,
total tannins decreased from 137.60 to 120.80 mg GAE/g dw with the *Botrytis* infection process (Ky et al. 2012). Major content variations between healthy and rotten grapes detected for all the phenolic compounds as shown by TPC, total tannin and anthocyanin contents which decreased markedly by 84, 70 and 82 per cent, respectively (Fig. 5).

Landrault et al. (2002) have reported the presence of viniferin (stilbene oligomers having chemopreventive activity) only in red and botrytized sweet white wines with levels between 0.1 and 1.63 mg/L. Nikfardjam et al. (2006) have reported higher levels of polyphenols in white botrytized wines as compared to non-botrytized white wines. The antioxidative capacities (TEAC values) for the German wines ranged from 0.6 to 2.8 mmol/L, while the wines from Tokaj showed much higher values ranging from 1.1 up to 10.8 mmol/L.

**Aromatic compounds**

Botrytized wines produce complex aroma compounds during the botrytization process and during vinification process these compounds can easily be transferred from skin to must. The characteristic aroma of wines made from *Botrytis* infested fruit is due to formation of 3-hydroxy-4, 5-dimethyl-2-furanone. These wines also possess higher level of other aroma compounds like homofuraneol, furaneol, norfuraneol, phenyl-acetaldehyde and methional (Sarrazin et al., 2007). A comparative analysis of wines obtained from manually selected healthy and botrytized grapes was carried out and aroma analysis revealed that most of the compounds varied significantly according to the percentage of botrytized berries utilized (Table 3). Botrytized wines contain few ethyl esters (C6-C10) and related fatty acids as well as more fruity acetates, such as Isoamyl and 2-phenethyl acetate than healthy wines. In addition, the specific volatile compounds like furfural, benzaldehyde, phenylacetaldehyde and benzyl cyanide are also synthesized by the fungus. The sotalone (3-hydroxy-4, 5-dimethyl-2 (5H) furanone) is one of the main compounds which has been found to cause the “*roti*” character in botrytized grapes. Negri et al. (2017) have reported the presence of various volatile benzenoids and benzaldehyde like vanillin, cresols, guaiacols and eugenol which are generally characterized by the sweet/spicy aroma notes.
Wine making from botrytized grapes

The wine making process from the healthy berries is already well established but due to various technological problems the production of botrytized wines is the challenging task for the wine-makers.

Harvesting of noble grapes

The harvesting period of the berries is prolonged as all the berries are not at perfect noble rot stage. So, the individual berries which are perfect for the preparation of botrytized wines are harvested mostly at pourri rotis stage which is termed as triage. Depending upon the climatic conditions, there may be three to four selective pickings in a year. The wine prepared from the selective harvest of individual berries is of superior quality. As the multiple harvesting is a costly procedure, so mostly single picking followed by separating botrytized berries is practiced.

Must preparation

The various steps followed for the preparation of must includes removal of deteriorated fruits, thermo-vinification, whole cluster pressing, separation of press fractions, cryoextraction, and post-fermentation heat treatment (Ribereau-Gayon et al. 1976). Sulfiting of juice is practiced during wine making process so as to avoid oxidation by inhibiting laccase, permit clarification, and inhibit acetic acid bacteria along with other antifungal substances. Light sulfiting (3-5 g/hl) of the juice has been recommended at this stage (Ribereau-Gayon et al. 2006). During juice extraction various polysaccharides like \( \beta \)-glucan may get released from the berry skin and can cause clarification problems in the must. This \( \beta \)-glucan forms a localized network between the epidermal cells and the pulp of the infected berries. In presence of ethyl alcohol the glucan chains gets aggregated and block the filters during wine making process.

In 1980s it was suggested that Trichoderma \( \beta \)-glucanase could be successfully used for clarification of juice from grapes infected with B. cinerea, where pectolytic enzymes were ineffective on \( \beta \)-glucans (Villettaz et al. 1984; Villettaz, 1990). As the cost of Trichoderma \( \beta \)-glucanases is high, settling and decanting processes for the juice clarification are generally practiced. Juice

<table>
<thead>
<tr>
<th>Aroma compound</th>
<th>W-100</th>
<th>W-80</th>
<th>W-60</th>
</tr>
</thead>
<tbody>
<tr>
<td>C6 alcohols</td>
<td>1656 ± 76</td>
<td>1855 ± 33</td>
<td>1691± 114</td>
</tr>
<tr>
<td>Other alcohols</td>
<td>13978 ± 1845</td>
<td>14233 ± 522</td>
<td>13593 ±366</td>
</tr>
<tr>
<td>Fermentative esters</td>
<td>7099 ± 309</td>
<td>8370 ± 913</td>
<td>8569 ± 929</td>
</tr>
<tr>
<td>Fatty acids</td>
<td>4422 ± 144</td>
<td>3985 ± 67</td>
<td>3663 ± 456</td>
</tr>
<tr>
<td>Aldehydes and ketones</td>
<td>79 ± 6</td>
<td>110 ± 10</td>
<td>114 ± 8</td>
</tr>
<tr>
<td>Terpenes</td>
<td>47 ± 1</td>
<td>58 ± 4</td>
<td>61 ± 2</td>
</tr>
<tr>
<td>C130-norisoprenoides</td>
<td>34 ± 1</td>
<td>42 ± 2</td>
<td>43 ± 2</td>
</tr>
<tr>
<td>Lactones</td>
<td>4403 ±146</td>
<td>4880 ± 276</td>
<td>5066 ± 461</td>
</tr>
<tr>
<td>Benzenoids</td>
<td>480 ± 38</td>
<td>501 ± 37</td>
<td>599 ± 41</td>
</tr>
<tr>
<td>Phenols</td>
<td>7 ± 0.3</td>
<td>7.2 ± 0.6</td>
<td>8.2 ± 0.6</td>
</tr>
<tr>
<td>Others</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-(3-methylbutyl)-acetamide</td>
<td>65 ± 3</td>
<td>915 ± 142</td>
<td>1878 ± 138</td>
</tr>
<tr>
<td>Not identified</td>
<td>1995 ± 179</td>
<td>2598 ± 369</td>
<td>3287 ± 80</td>
</tr>
<tr>
<td>Total</td>
<td>34265 ± 1865</td>
<td>37554 ± 1629</td>
<td>38563 ± 550</td>
</tr>
</tbody>
</table>

Source: Fedrizzi et al. 2011.
can be clarified within 3-4 days by natural settling process at 0 °C (Ribereau-Gayon et al. 2006).

**Fermentation**

*B. cinerea* infection encourages the proliferation of acetic acid bacteria (Joyeux et al., 1984) and yeasts like *Kloeckera apiculata* and *Torulopsis stellata* (Le-Roux et al. 1973) on grapes which in turn can affect the chemical composition and microbial makeup of the must/juice. The yeasts like *Saccharomyces* species contributes significantly to the alcoholic fermentation process. Alcohol tolerant strains of *Saccharomyces cerevisiae* in practice, are preferred to *Saccharomyces bayanus* because these strains are less affected by the botryticine (group of hetero-polysaccharides) released by *B. cinerea* during the infection process. *Saccharomyces uvarum* has also been isolated from botrytized wines by various workers (Antunovics et al. 2003; Magyar et al. 2008; Tosi et al. 2009). Small wooden barrels are traditionally used for the fermentation of the botrytized wines. Stainless steel tanks are now widely used for the fermentation process of botrytized wines but in Sauternes and Tokaj wooden barrels are still in use (Magyar, 2011). Musts fermented in barrels produce more alcohol than the musts fermented in large fermentors as the fermentations stops earlier in the tanks. In most of the winery the temperature for the fermentation process is kept at 20 and 20-24 °C in Tokaj and Sauternes regions, respectively (Magyar, 2010 and Ribereau-Gayon et al. 2000). The nutrients are depleted by the fungus and this is one of the limiting factors in fermentation process of botrytized wines. A significant difference in available nitrogen content in healthy must (182 mg/l) and botrytized must (84 mg/l) have been reported by Bely et al. (2008). Dubourdieu (1999) have recommended addition of thiamin (0.6mg/ l), diammonium phosphate (300 mg/l) and active dry yeast (10-15 g/l) so as to ensure optimum fermentation rate. As soon as sufficient alcohol content is achieved, fermentation is recommended to be stopped without delay so as to control the volatile acidity in wine. The fermentation of sweet white wines is stopped either by pasteurization or by the use of 20-30 g/hl of SO₂ (Ribereau-Gayon et al., 2006). A new approach of using Dimethylidicarbonate (DMDC) has been considered as a suitable inhibitor for ceasing fermentation. A concentration of 100-200 mg/l DMDC has been found effective in ceasing fermentation process (Magyar, 2011). During maturation gluconic acid content is more than 600 mg/L, heterolactic fermentations appear with certain intensity, producing high concentrations of lactic acid and volatile acidity (upto 2 g/L), and the latter is known to affect the quality of the wine. To control this increase in volatile acidity *Torulaspora delbrueckii* can be used during fermentation as mixed culture with other strains (Bely et al. 2008; Renault et al. 2009). The premium *Sauternes* wines are aged in barrels for 12-18 months and sometimes up to a period of 2 years or more. Whereas, barrel-aging of the German wines is avoided so as to lower down the risk of re-fermentation as they are having low alcohol content (Ribereau-Gayon et al. 2000).

**CONCLUSION**

Botrytized wines are natural sweet wines produced from the withered and shrivelled berries and *B. cinerea* plays the key role in their production. This fungus is widely used for the production of botrytized wines like *Sauternes*, Tokaji Aszu (Fordias, Malslas and Szamorodni) and Trockenbeeren Auslese. *B. cinerea* is responsible for the induction of various physico-chemical changes in the berries or must which ultimately affects the quality of wine produced. *B. cinerea* pathogenesis or botrytization process is a complex procedure due to various chemical and enzymatic interactions of fungus with the grape berries. These interactions are needed to be studied further with the approach of newer techniques. Induced botrytization process is a significant area of further research in the regions (hot and dry) where conditions for the development of noble rot are not favorable. *In vitro* establishment procedures of *B. cinerea* for the production of botrytized wines with the addition of inoculum into the juice or must rather than in the grape berries are further needed to be refined with help of advanced technologies and
better fermentation techniques. Studies on selection or suitability of other yeast species or strains in the fermentation process needs to be explored for the further improvements in the production of botrytized wines.

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