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REVIEW PAPER

Botrytized Wines: A Review

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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, phenylacetaldelyde 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 cosumers.

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, arabitol, 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; Thudichhum 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 ft^2 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

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

Component	Sauvignon berries		Semillon berries		Germany		Tokaj	
	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected
Fresh weight/100 berries (g)	225	112	202	98	85	36	—	—
Sugar content (g/liter)	281	326	247	317	295	500	685	708
Acidity (g/litre)	5.4	5.5	6.0	5.5	15.20	20.80	16.55	14.70
Tartaric acid (g/litre)	5.2	1.9	5.3	2.5	2.60	2.40	4.81	4.44
Malic acid (g/litre)	4.9	7.4	5.4	7.8	8.00	10.10	5.82	7.42
Citric acid (g/litre)	0.3	0.5	0.26	0.34	0.20	0.24	0.11	0.99
Gluconic acid (g/litre)	0	1.2	0	2.1	1.50	2.17	3.20	3.88
Ammonia (g/litre)	49	7	165	25	—	—	—	—
pH	3.4	3.5	3.3	3.6	—	—	—	—

Source: *Charpentie, 1954; Ribereau-Gayon et al. 1980; Linssen, 1986; Dittrich, 1989 and Magyar, 2006.*

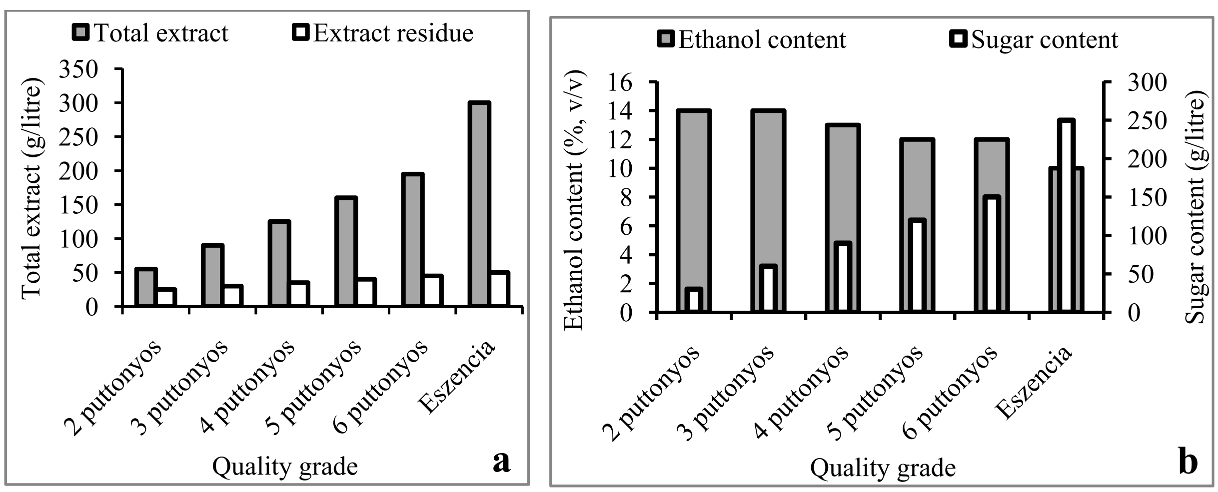

Fig. 1 (a and b): Chemical composition of Tokaji wines (Based on Farkas, 1988)

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), *Beerenausleen* wines and *Trockenbeerenausleen* wines. Out of these three *Beerenausleen* and *Trockenbeerenausleen* wines are made from *B. cinerea* attacked berries which possess higher sugars content. These wines have relatively 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

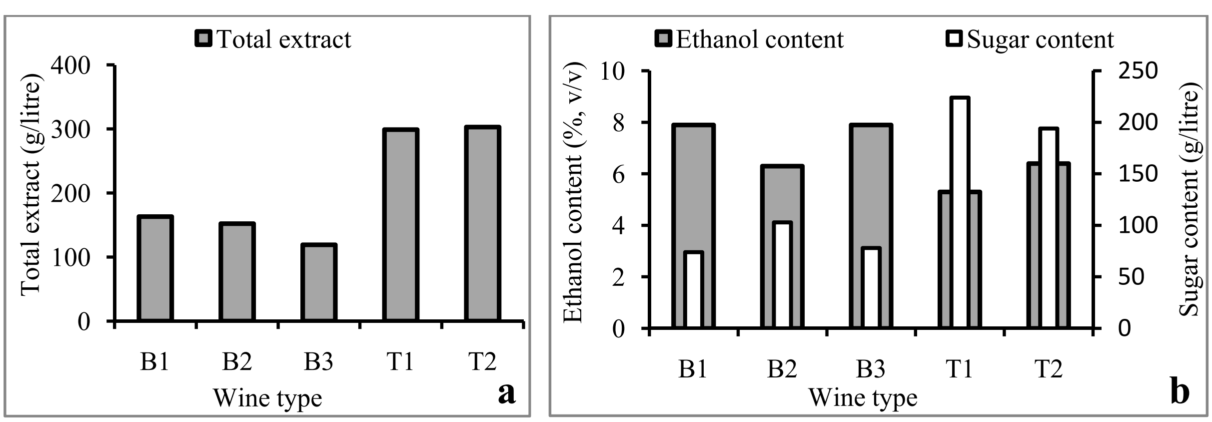

Fig. 2 (a and b): Chemical composition of *Beerenauslesen* (B1, B2, B3) **and** *Trockenbeerenauslesen* (T1, T2) wines (Based on Watanabe and Shimazu, 1976) of host epidermis (Williamson *et al.* 1995). After attachment and germination of conidia the host tissue invasion takes place by means of active 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

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

Source: *Thakur and Sharma, 2011.*

Bulit and Lafon (1970) have reported the mean temperature range of 15-20 ^{o}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 hyphe powder of *B. cinerea* dissolved in 20 g/l glucose with spore concentration of 1×10^6 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 (Villettaz *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,

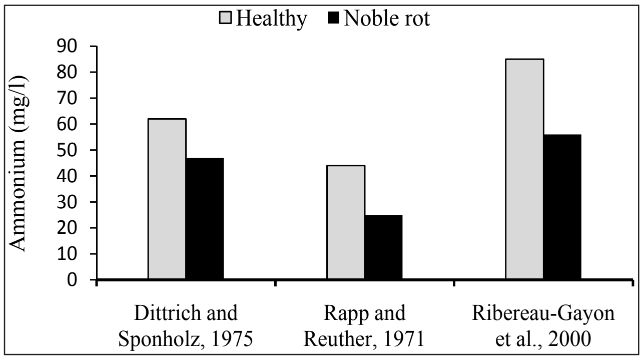

Fig. 3: Effect of *Botrytis cinerea* on ammonium compound of grape juice (Based on Magyar, 2011)

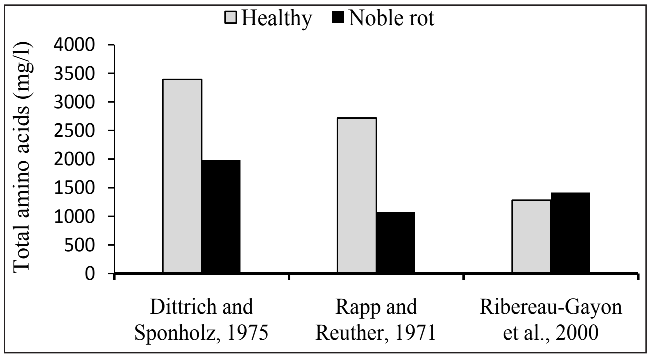

Fig 4: Effect of *Botrytis cinerea* on total amino acids of grape juice (Based on Magyar, 2011)

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).

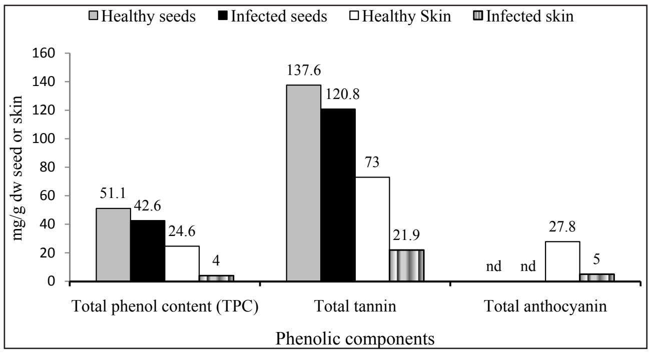

Fig. 5: Concentration of phenolic components in seeds and skins of healthy grapes and botrytized grapes (Based on Ky *et al.* 2012)

nd: not detected

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, phenylacetaldelyde 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.

Table 3: Aroma compounds in wines prepared from must from healthy grapes (W-100) and botrytized grapes in different proportions (W-80 and W-60)

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

Source: *Fedrizzi et al. 2011.*

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 β-glucan may get released from the berry skin and can cause clarification problems in the must. This β-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* β-glucanase could be successfully used for clarification of juice from grapes infected with *B. cinerea*, where pectolytic enzymes were ineffective on β-glucans (Villettaz *et al*. 1984; Villettaz, 1990). As the cost of *Trichoderma* β-glucanases is high, settling and decanting processes for the juice clarification are generally practiced. Juice 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/hl) 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 SO_2 (Ribereau-Gayon *et al.,* 2006). A new approach of using Dimethyldicarbonate (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 delbtueckii* 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 upto 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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