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The effect of black rot on grape berry composition

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ABSTRACT

Guignardia bidwellii, indigenous to North America, is a significant pathogen of grapes long known in Hungary, infecting only the growing green parts of the vine (leaves, petioles, shoots, and bunches). In the absence of adequate plant protection and extreme weather conditions such as a predominantly humid, warm year, black rot of grapes can be expected. The pathogen can cause high yield losses due to grape rot and reduce wine quality if the infection is severe.

The evolution of certain biogenic amine compounds were investigated under the influence of grape black rot. The results obtained showed that they were present in low concentrations from an oenological point of view. Polyphenol composition was consistent with the literature, blackening affected mainly the concentration of catechin. Black rot fungus does not produce β-glucosidase enzyme. In terms of resveratrol content, black rot has no particular effect. However, like *Botrytis cinerea*, it produces glycerol and, proportionally, gluconic acid in lower concentrations.

It can be concluded that black rot of grapes does not cause health problems when introduced into wine processing.

KEYWORDS

black rot, *Guignardia bidwellii*, chemical composition, polyphenols, resveratrol, biogenic amines

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1. INTRODUCTION

Economically the most significant pathogens of grape are downy mildew (*Plasmopara viticola*), powdery mildew (*Erysiphe necator*), grey rot (*Botrytis cinerea*), black rot (*Guignardia bidwellii*), and other local or temporarily occurring pathogens (Fischer and Kassemeyer, 2003). Black rot and downy mildew are the two major diseases of cultivated grape (*Vitis vinifera*) worldwide. Black rot is caused by *G. bidwellii,* a fungus originating from North America, responsible for one of the highest financial losses regarding Eurasian grape. The main *V. vinifera* varieties in production are either moderately or highly susceptible to the disease, depending on the production area and phenological phase (Jabco et al., 1985).

This study aimed to identify the changes caused by black rot in the composition of the berry. The most important parameters of the berries of grapes affected by black rot were investigated. Polyphenols, as one of the most important groups of compounds from the oenological aspect, were examined, as well as organic acids, sugars, resveratrol, and histamine.

Polyphenols have an essential role in plant development and reproduction, protecting against biotic and abiotic stresses, such as the effect of pests and diseases, UV radiation, and damage to the plant (Winkel-Shirley, 2002). Polyphenols have antioxidant (Landrault et al., 2001) and anti-inflammatory (Castilla et al., 2006) effects; they are beneficial for the heart (Zern and Fernandez, 2005) and the nervous system (Shukitt-Hale et al., 2006), and can be used in cancer prevention (Castillo-Pichardo et al., 2009). Phenolic compounds are responsible for the bitter taste, contracting mouthfeel, and browning of wines. Flavonoids have reducing and antioxidant properties and are often polymerised (Nagy et al., 2017).

Biogenic amines in food are derived from decarboxylation of amino acids through the activity of exogenous enzymes released by various microorganisms (Erdag et al., 2018). Biogenic amines are low molecular weight aliphatic, alicyclic, or heterocyclic nitrogen-containing compounds, and they are essential in human body. They are natural components of different foods and play a role in shaping the flavour of food. They can be found in grape must but can also be formed during alcoholic and malolactic fermentation and wine ageing (Guo et al., 2015).

2. MATERIALS AND METHODS

2.1. Samples

Six different grape varieties were tested for their black-rotten berries. The analysis was based on the current practice of visual inspection of berries that were considered to affect by black rot. Samples were resistant white grape varieties Bácska, Danubius, Hibernál, Palatina, and Panonija collected from Borota and Kékfrankos varieties collected from Villány. After representative hand sampling, only fully black-rotten, mummified berries from infected bunches were sorted and used for sample preparation.

2.2. Methods

2.2.1. Spectrophotometric methods. Determination of sugar, glycerol, gluconic acid, malic acid, lactic acid, citric acid, and tartaric acid was performed by a Thermo Scientific Gallery desktop discrete photometric analyser.

Sugar, glycerol, gluconic acid, malic acid, lactic acid, and citric acid were examined by enzymatic reaction and tartaric acid was determined by a colour reaction.

Galacturonic acid was analysed by Megazyme Ltd. D-Glucuronic/D-Galacturonic Acid Assay Kit. It is a simple, reliable, and accurate method based on an enzymatic reaction with a limit of detection (LOD) of 15.5 mg L^{-1}. The wavelength of the spectrophotometric measurement is 340 nm.

Spectrophotometric procedure was performed with a MOM Spektromom 195 device. Total polyphenol was determined with Folin-Ciocalteu-phenol reagent expressed as gallic acid equivalent (Kállay et al., 1999). The amount of leukoanthocyanin was determined with spectrophotometer after heating with a 40:60 mixture of hydrochloric acid-butanol containing iron(II) sulphate according to the modified method of Flanzy et al. (1970). Its concentration is expressed in malvidin-3,5-diglucoside equivalents. Catechin was analysed in alcohol-diluted wine using vanillin sulphuric acid at 500 nm by spectrophotometry (Rebelein, 1965).

2.2.2. High-Performance Liquid Chromatography (HPLC) methods. Determination of grape berry extracts was performed by Shimadzu LC-20 HPLC. The system consisted of a dual-pump module LC-20AD, a DGU-20A5R mobile phase degasser, an autosampler SIL-20AHT, a CTO-20A column oven with an FCV-12AH high-pressure six-port switching valve, and a fluorescence detector RF-20AXS and a diode array detector SPD-M20A. The HPLC system was controlled by a CBM-20A communication module. Lab-Solution software (Shimadzu Corporation) was used for the data acquisition and evaluation.

Grape berries were extracted for HPLC analysis as follows: 20 g sample was added into a blender jar, 7.2 mL methanol and 52.8 mL water were added (providing 60 mL of 12 v/v% methanol). The blender jar was covered and the sample was blended at high speed for 1 min. After 30 min, the sample was centrifuged for 2 min. The supernatant was extracted with 2 × 50 mL chloroform. After evaporation, the residue was dissolved in 2 mL HPLC eluent. These berry extracts were injected into the chromatograph.

Organic acids (shikimic acid, succinic acid, and fumaric acid) were separated and simultaneously determined by high-performance liquid chromatography (HPLC) with a method by the International Organisation of Vine and Wine (OIV, 2004).

For the determination of caftaric acid, catechin, and epicatechin a method of Larrauri et al. (2017) was used.

For liquid chromatographic determination of resveratrol, the method of Kállay and Török (1997) was used. The separation column was RP-C18 (100 × 4.6 mm, 5 µm). The mobile phase was a mixture of acetonitrile: methanol: water (5 : 5 : 90, v/v/v) at a flow rate of 2 mL min^{-1} in isocratic elution. The temperature of the column was set at 30 °C, and the detection was at a wavelength of 306 nm.

Determination of biogenic amines (BAs) by HPLC was performed on an HP 1050 chromatograph with an HP 1046A fluorescence detector. Mobile phase was 0.08 M acetic acid (A)

and acetonitrile (B) at a flow rate of 1 mL min^{-1} in gradient elution (Table 1) (Kállay and Nyitrai-Sárdy, 2003; Nyitrai-Sárdy et al., 2017; Oláhné Horváth et al., 2020; Nagy et al., 2021).

Grape musts were filtered with a 0.45 µm filter before the analysis.

2.3. Statistical analysis

The assessment was performed using one-way ANOVA in Microsoft Excel 2016 to determine the significant difference between samples (Freedman, 2005). Results were obtained at the 95% significance level.

3. RESULTS AND DISCUSSION

To study the effect of *G. bidwellii* on the berry composition of black rot affected grapes, berries were thoroughly analysed.

Table 2 shows sugar, glycerol, gluconic acid, and galacturonic acid contents of the berries.

Black rot caused an increase in sugar concentration. Just like in the case of *B. cinerea* infestation, glycerol is produced in the berries, as well as gluconic acid, although in a lower concentration. The galacturonic acid level suggests pectinase enzyme activity. Table 3 shows the organic acid contents of the samples.

Table 1. Elution gradient

Time (min)	A%	B%
3.5	70	30
10	35	65
21	28	72
22	20	80
25	20	80
30	70	30

A: 0.08 M acetic acid; B: acetonitrile.

Table 2. Sugar, glycerol, gluconic acid, and galacturonic acid contents of black rot affected berries

Grape variety	Sugar (g kg^{-1})	Glycerol (g kg^{-1})	Gluconic acid (g kg^{-1})	Galacturonic acid (mg kg^{-1})
Palatina	660.4	14.8	1.6	600
Panonija	586.8	15.2	1.4	1,368
Hibernál	534.5	11.2	1.5	600
Danubiusz	631.0	9.6	1.4	550
Bácska	620.6	14.4	1.2	1,080
Kékfrankos	616.0	33.2	1.3	600
Control	615.3	n.d.	n.d.	n.d.

n.d.: not detected.

Table 3. Organic acid contents of black rot affected grape berries

Grape variety	Tartaric acid (g kg^{-1})	Malic acid (g kg^{-1})	Shikimic acid (mg kg^{-1})	(+)-Lactic acid (g kg^{-1})	Citric acid (mg kg^{-1})	Succinic acid (g kg^{-1})	Fumaric acid (mg kg^{-1})	Caftaric acid (mg kg^{-1})
Palatina	8.2	n.d.	n.d.	0.056	n.d.	6.5	125.4	80.0
Panonija	12.4	n.d.	n.d.	0.063	n.d.	3.4	369.1	108.1
Hibernál	11.2	n.d.	n.d.	0.064	n.d.	6.7	110.8	111.9
Danubiusz	15.9	n.d.	n.d.	0.066	n.d.	6.4	161.2	108.3
Bácska	15.6	n.d.	n.d.	0.100	n.d.	6.6	104.8	120.5
Kékfrankos	14.3	n.d.	n.d.	0.076	n.d.	9.2	78.0	80.6
Control	10.3	7.2	n.d.	0.003	0.02	n.d.	n.d.	90.2

n.d.: not detected.

Malic acid was non-detectable, while (+)- lactic acid was found in berries. Furthermore, shikimic and citric acids were absent in the samples, while a considerate amounts of succinic and fumaric acids were measured.

Table 4 shows the polyphenol content of samples. The average total polyphenol content of white wines is 170–300 mg L^{-1}, and 4,000 mg L^{-1} of red wines (Bene and Kállay, 2019). Samples were characterised by the ratio of catechin and epicatechin. Normally a higher epicatechin level is typical during ripening, then 1:1 ratio develops at the fully ripened stage. Increased catechin concentrations were found in black rot infected berries.

Table 5 shows the resveratrol levels of the samples. As expected, only resveratrol glucosides were detected in the samples. The black rot-causing fungus does not produce any β-glucosidase enzyme, unlike in the case of *B. cinerea*. *cis*-Resveratrol was measured in Kékfrankos (Blue frank) that is an atypical result. According to our findings, black rot does not affect the resveratrol levels in berries.

In this study we have measured amines that can be characterised with a proven health effect. From the wine production point of view the amine concentrations were low. Table 6 shows the biogenic amine concentration of samples. A significant amount of histamine was detected in the samples. Lower histamine levels and a steady amount of melatonin, tyramine, and serotonin were measured in the shrivelled berries, while these values were higher in the black rot infected berries.

The investigation and separation of the microbial composition of berries affected by black rot were not the aim of the study. No further studies have been carried out in this context.

Table 4. Polyphenol contents of black rot affected grape berries

Grape variety	Total (mg kg^{-1})	(+)-Catechin (g kg^{-1})	(−)-Epicatechin (g kg^{-1})	Leucoanthocyanins (procyanidins) (mg kg^{-1})	TAC (mmol kg^{-1})
Palatina	5,932	3,763	924	3,276	112.4
Panonija	5,936	3,640	448	3,476	112.4
Hibernál	6,176	3,452	1,156	3,372	117.2
Danubiusz	5,328	4,332	176	3,992	100.8
Bácska	6,480	4,744	100	3,864	122.8
Kékfrankos	6,364	5,796	120	5,180	120.8
Control	5,899	3,826	256	3,825	111.8

TAC: Total Antioxidant Capacity.

Table 5. Resveratrol levels of black rot affected grape berries

Grape variety	*cis*-Piceid (mg kg^{-1})	*trans*-Piceid (mg kg^{-1})	*cis*-Resveratrol (mg kg^{-1})	*trans*-Resveratrol (mg kg^{-1})
Palatina	n.d.	0.28	n.d.	n.d.
Panonija	n.d.	0.38	n.d.	n.d.
Hibernál	n.d.	0.93	n.d.	n.d.
Danubiusz	0.46	0.33	n.d.	n.d.
Bácska	n.d.	0.31	n.d.	n.d.
Kékfrankos	n.d.	3.28	0.91	n.d.
Control	n.d.	n.d.	n.d.	n.d.

n.d.: not detected.

Table 6. Biogenic amine concentrations of black rot affected grape berries

Grape variety	Tyr (mg kg^{-1})	Ser (mg kg^{-1})	His (mg kg^{-1})	Mel (mg kg^{-1})
Palatina	0.07	0.05	11.6	n.d.
Panonija	0.06	0.07	10.8	n.d.
Hibernál	0.09	0.08	8.8	0.02
Danubiusz	0.10	0.06	9.8	0.01
Bácska	0.08	0.04	10.5	n.d.
Kékfrankos	0.11	0.10	11.2	0.12
Control	0.04	0.06	3.2	n.d.

Tyr: Tyramine, Ser: Serotonin, His: Histamine, Mel: Melatonine.
n.d.: not detected.

4. CONCLUSIONS

This study aimed to identify the changes caused by black rot in the chemical composition of grape berry.

The changes caused by black rot are negligible in terms of the grape's nutritional values and undesirable compounds such as histamine. Although melatonin, tyramine, and serotonin are produced in the berry, their levels are negligible.

According to the chemical composition data obtained from black rot affected grapes, the chemical composition of wine will not be altered in terms of polyphenols, biogenic amines, and resveratrol. Botrytis produces less histamine than black rot. Wine treatment processes such as bentonitic maceration or filtration reduce the histamine concentration, thus there is no risk of histamine contamination of wine.

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