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Original paper

Organic acids and sugars profile of some grape cultivars affected by grapevine yellows symptoms

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Abstract Grapevine yellows are a group of infectious diseases, which are usually associated with phytoplasma pathogens, affecting quality of grapes and survival of grapevine. The objective of this work was to evaluate sugars, total phenols and organic acids concentrations in the grapes of four wine cultivars (Fetească Neagră, Pinot Noir, Traminer rose, Chardonnay) healthy and affected by specific symptoms to phytoplasmoses. Berry samples were collected from the Miniș-Măderat (Arad County) and Odobești (Vrancea County) vineyards. Individual sugars and organic acids compounds were identified and quantified in grape berries by the high performance liquid chromatography (HPLC) method using DAD and RID detectors, while total phenolic compounds by using spectrophotometry. The content and composition of sugars and acids varied between healthy and symptomatic plants. Analysis of grape juice revealed reduction in sugars (fructose and glucose) from symptomatic to non-symptomatic vines. Lower values of acidity were obtained in grapes of affected compared to non-affected vines. The differences in acids content between grapes of affected and not affected vines were more pronounced at Pinot Noir variety, with lower concentrations of malic and tartaric acids in grapes from symptomatic vines in both locations studied. The affected grapes did not differ significantly from the healthy ones, regarding to succinic acid content. A notable increase of total phenolic compounds was observed in symptomatic compared to asymptomatic grape berries. The results of this paper showed a clear negative effect of the phytoplasmoses symptoms on grape fruits composition.

Keywords Grape quality, phenolics, biotic stress, phytoplasmoses.

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Introduction

Grapevine (*Vitis vinifera* L.) is cultivated all over the world and plays an important role in the economy of many countries, for fresh consumption, wine, juices and raisins production (MARGARIA & al, 2014[1]; NAIDU et al, 2014 [2]). The quality of grapes for winemaking is affected by various factors, such as pedo-climatic, agricultural management and pest and diseases. During the ripening phase, a number of major physiological and biochemical changes occur simultaneously in the grape berries, and these changes determine the quality of the fruit at harvest (RUSJAN & al, 2008 [3]; ALI & al, 2008 [4]; EYDURAN & al, 2015 [5]).

Grapevine yellows diseases, also called phytoplasmoses, are a complex of infectious diseases widespread in viticultural areas around the world. Their causal agents are phytoplasmas - bacteria without cell walls that multiply in diseased plants and insects sucking in sieve elements of plants (WEINTRAUB & BEANLAND, 2006 [6]; ZAHAVI & al, 2013 [27]). Two phytoplamoses, flavescence dorée and bois noir, have the greatest economic impact on viticulture in Europe. From the economical and oenological point of view, the impact of phytoplasma infections can have dramatic consequences for vine growers and wine producers because drastically reduce yield and affect grapes chemical composition (BERTAMINI & al, 2002 [7]; GARAU & al, 2007 [8]; ENDESHAW & al, 2012 [9]; RUSJAN & al, 2012a, 2012b [10, 11]; EMBER & al, 2016 [12]). Berry dehydration in diseased cultivars was associated with phytoplasma infection, suggesting that this caused inhibiting of sugar transport, poor synthesis of anthocyanins, and the lack of organic acid degradation (MATUS & al, 2008 [13]). Grapevine plants are sensitive to phytoplasma infection, and symptoms range from very severe, as in high susceptible cultivars, to barely visible, as in tolerant ones.

In Romania, the first symptoms on grapevine related to the yellows diseases were observed from 1969 (RAFAILA & COSTACHE, 1970 [14]. The studies that followed then reported the spreading of diseases symptoms and natural vector species in vineyards (CHIRECEANU & al, 2011 [15]) and the presence of phytoplasma pathogens associated with *stolbur* group disease (PLOAIE & CHIRECEANU, 2012 [16]; CHIRECEANU & al, 2013 [17]). To the best of our knowledge there are not reports on the impact of phytoplasma on grape composition in Romania and this is the first study to characterise wine grape affected by phytoplasma symptoms.

The aim of this work was to evaluate the chemical profile in terms of major sugars, acids and total phenolic compounds of different wine grape cultivars in relation to specific symptoms to phytoplasmoses.

Material and Methods

1. Plant material

Four wine grape cultivars (Fetească Neagră, Pinot Noir red varieties, Traminer rose variety, Chardonnay white variety) from the vineyards Miniş-Măderat located in Arad County (Western Romania) and Odobești located in Vrancea County (Eastern Romania) were studied in two successive years (2016–2017). Samples consisting of 100 berries were collected during harvesting period, from vine plants that had never shown symptoms (healthy) and vine plants that showed symptoms of phytoplasmoses (diseased). Both vineyards were monitored each year for phytoplasma symptoms and Bois Noir phytoplasma was detected (unpublished data). The vines considered 'diseased' showed severe symptoms characteristic to the infections with phytoplasma pathogens, such as the yellowing of foliage on white grapevine cultivars or reddening on red grapevine cultivars, smaller and rolled downward leaves with ribs affected by necrosis, the annual shoots flexible than normal, grapes with shrivelled berries.

2. Extraction and sample preparation

The extraction of carbohydrates and organic acids from fresh grape juices followed the method of LEE et al, 2009 [18]. The mixture of about 100 whole berries (skin, pulp and seeds) from each replicate were crushed with a hand blender for 5 min, then centrifuged for 15 min at 5000 rpm. The resulting supernatants were filtered using a 0.45-μm filter membrane (Millipore Millex-HV Hydrophilic PVDF, Millipore, USA) before running HPLC. The grape berries were extracted in 96% ethanol containing 0.01% butylated hydroxytoluene (BHT) for 4 hours. The extract was filtered again prior to injection in HPLC system for determination of total phenols. Three replicates were performed, each replicate was extracted and analyzed separately.

3. Detection and HPLC analysis of sugars and organic acids

In the present study, all chemicals and solvents used were analytical and HPLC grade. Organic acids standards (tartaric acid, citric acid, malic acid, acetic acid, succinic acid) were purchased from Sigma-Aldrich (St louis, MO; USA). Glucose, fructose and sucrose standards were obtained from Merck (Darmstadt, Germany). Standard solutions were prepared individually in distilled water or in ethanol and then filtered. Samples and standards were injected three times and average values were calculated.

Samples of grape juice were diluted with mobile phase in 1:1 ratio before injected into the system. So make sure a good homogenization of the sample in the mobile phase structure in order to avoid precipitation between mobile phase and sample. The mixtures obtained were filtered again through a 0.45 µm membrane filter prior to injection in HPLC system.

An Agilent Series 1260 HPLC system (Agilent Technologies, Palo Alto, CA, USA) was used for the HPLC-DAD analysis. The HPLC system consisted of a degasser, an isocratic pump system, an autosampler, a thermostatted column compartment, a refractive index detector (RID) for sugar analysis, and a diode array detector (DAD) was monitored at 210 nm for the analysis of organic acids. Sugars and organic acids were simultaneously analyzed using a C18 column (250 × 4.6 mm) and maintained at 45°C during analysis. The analytical conditions used were as follows, flow rate of mobile phase 1 ml min^{-1}, the injection volume was 50 µL, eluent was acetonitrile: deionized water 75:25 (v/v). The chromatographic peaks corresponding to each sugar and organic acid were identified by comparing the retention times and spectra with those of standards. Sugars and organic acids content was expressed as g L^{-1} of juice. Total phenolic acids were quantified as caffeic acid (320nm) and total flavanols as catechin (280nm) and expressed in mg L^{-1}.

4. Statistical analysis

Statistical analysis was performed using the statistic package of Microsoft Office 10.

Results and Discussions

Phytoplasma infections are important diseases affecting table and wine grape cultivars, but little is known about their effect on grape composition and wine quality (BERTACCINI & DUDUK, 2010 [2]; RUSJAN et al, 2012b [11]; EMBER & al, 2016 [12]; EMBER et al, 2018 [20]). The presence of symptoms associated with these diseases can alter grape composition, change in their sugars, acids and phenolic profiles that contribute greatly to the taste and final alcohol content of wine.

The results obtained on wine grape cultivars studied during two successive years, show that the content and composition of sugars and acids varied between genotypes, and healthy and symptomatic cultivar (table 1 and figures 1 and 2).

Data in table 1 shows that the amount of fructose and glucose decreased because of the presence of phytoplasmoses. The glucose content was higher than the fructose content in all analyzed cultivars. Results of SENSOY & al, (2015) [21] and EYDURAN & al, (2015) [5] establish also that glucose content is much higher comparative with fructose content in grape berry. Sucrose content was very low even none detectable in all cultivars studied. The ratio of glucose/fructose in grape berries varied in ranged from 1.0 to 1.06. There is no difference in this ratio between healthy samples and those affected by yellows. Also, research by EMBER & al, (2016) [12] showed lower soluble solids content may have occured in Chardonnay grape fruit BN-affected. Similar negative effects have been observed in vine affected by virus infections that recorded decrease of yield and change of grape composition (MARTINEZ & al, 2016 [10]).

Table 1. Concentration of fructose, glucose, total sugars, total flavanols and total phenolic acids in berries of grapevine cultivars studied

Cultivars	Fructose g/L	Glucose g/L	Glc/Fru	Total sugars g/L	Total flavanols mg/L	Total phenolic acids mg/L	Total phenols mg/L
Pinot Noir healthy	57.06 ±0.12	58.36±0.10	1.02	115.42	62.40±0.10	1.06±0.14	63.46
Pinot Noir affected	44.19±0.31	44.67±0.21	1.01	88.86	65.40± 0.07	2.06±0.09	67.46
Traminer healthy	36.86±0.43	36.97±0.38	1.00	73.73	64.28±0.09	3.98±0.21	68.26
Traminer affected	32.20±0.40	33.82±0.25	1.05	66.01	64.84±0.08	3.82±0.19	68.66
Fetească Neagră healthy	51.81±0.09	55.33±0.12	1.06	107.14	58.08±0.13	5.52±0.11	63.60
Fetească Neagră affected	42.54±0.08	44.78±0.17	1.06	87.33	58.60±0.11	9.92±0.12	68.52
Chardonnay healthy	39.21±0.24	39.64±0.18	1.01	78.85	63.60±0.07	1.22±0.08	64.82
Chardonnay affected	36.61±0.44	37.42±0.26	1.02	74.02	65.72±0.09	2.56±0.07	68.28

The data are expressed as means ±SDV (standard deviation) of three replicates of each experiment

The total sugars concentration is an indicator of fruit maturity stage and indicates the potential alcoholic grades after fermentation and quality of wine (RUSJAN & al, 2008 [3]; EMBER & al, 2018 [20]). The total sugars concentration was lower for white cultivars compared to those of red cultivars. In case of Pinot Noir and Feteasca Neagra red cultivars the average content of total sugars decreased by 20% in affected vines as compared to healthy vines. However, in the Chardonnay white cultivar and Traminer rose cultivar the average values of total sugars decreased by 10% in phytoplasma infected vines in both years, as compared to healthy ones. This results are in accordance with those of ENDESHAW & al, 2012 [9] that observed a low total sugars content of grape berries as a consequence of Bois noir phytoplasma infection. In general, when comparing red cultivars with white cultivars the total sugars were higher in the red to the white ones.

The phenolic compounds in grapevines may considerably influence the taste (bitterness and astringency) of wine (PINELO & al, (2006) [23]. Flavonols are one of the phenolic compounds involved in the co-pigmentation phenomena in red wines and have been identified as having important antioxidant activity. Flavanols are responsible for bitterness in wine and play a role in astringency. These compounds are responsible for the browning reaction of the white wines and may have a negative impact on the sensory properties of these wines associated with bitter and astringent.

The total phenolic acids and total flavanols content in grape berries from symptomatic and non-symptomatic vine plants are presented in table 1. We observed an increase of total phenolic acids and total flavanols in symptomatic berry compared to healthy ones in all four cultivars studied. Also, it can be observed that the total phenolic acids content was lower than total flavanols content in both healthy and affected cultivars. These results are in agreement with those given by RUSJAN & al, (2012b) [11].

Infection with phytoplasma showed increased of total flavanols content, their total amount reached 65.40 mg mL^{-1} or 65.72 mg mL^{-1} compared to 62.4 mg mL^{-1} or 63.60 mg mL^{-1} in berries of Pinot Noir, respectively Chardonnay from uninfected plants. However, with regard to the Feteasca Neagra and Traminer cultivars, the total flavanols content does not differ between infected samples to the healthy ones. Similar results were reported by RUSJAN et al, (2012b) [11] who indicated that infection with Bois noir disease leads to the accumulation of flavanols and hydroxycinnamic acids in affected shriveled berries compared to unaffected berries of Chardonnay cultivar, which may have negative effects on wine quality.

Our data are also consistent with studies of MARGARIA et al, (2014) [1] that have revealed an increase of phenolic compounds in phytoplasma infected grapevine. However, our results are contradictory with those of LEE & MARTIN, (2009) [3] which supported that total polyphenolic content of whole berries was lower in infected vines with GLRaV-2 viruses compared to healthy one. Also, other authors LATTANZIO & al, 2006 [25] and TAWARE & al, (2010) [26] reported that grape berries infected by fungal diseases showed a high phenolic compounds concentration. The presented results are in accordance with the previous report of Ember & al, 2018 [20] that revealed elevated hydroxycinnamic acids (cafftaric and caffeic acids) and low flavonoid content (catechin and epicatechin) in grape affected by “Bois Noir” disese.

The results obtained for individual organic acids (tartaric, malic, citric, succinic and acetic acids) in helthy and affected berry by phytoplasmoses are presented in figure 1 and 2. Also, the HPLC chromatograms of organic acids and sugars in the grape juice sample for two cultivars, Feteasca Neagra and Traminer rose healthy and affected by phytoplasmoses, are illustrated in figure 3 and 4.

For organic acids there is a specific behavior to each acid type and also depending on the cultivar. The results showed variations in their concentration between healthy and affected grape for all cultivars. Thus, the concentration of tartaric, malic, citric and acetic acids decreases, while succinic acid concentrations increases in sample affected by phytoplasma symptoms. In all grape samples from vines affected by phytoplasmoses, except for Chardonnay, the concentration of tartaric and acetic acid was lower than in grapes from healthy vines. Except for Traminer rose cultivar where the concentration of citric acid increase in grapes affected by phytoplasma symptoms, in all other cultivars with symptoms content of this acid decreases. The data show that tartaric and malic acids are presented in large amounts compared to other acids in all cultivars studied.

The report of GARAU et al. (2007) [8] presented that pH and quantity of tartaric acid (g/L) in the must are not affected by the Bois noir disease. Our results showed differently from those of ENDESHAW et al (2012) [9] who found a slight increase of titratable acidity and also malic acid in grapevine with ”Bois noir” symptoms.

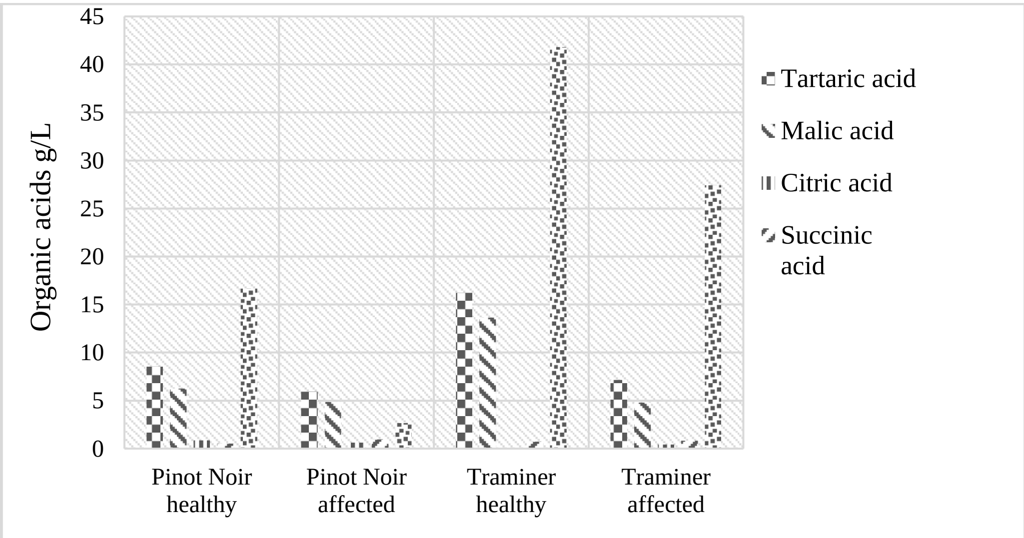

Figure1. Organic acids concentration in Pinot Noir and Traminer grapevine cultivars with and without phytoplasmoses symptoms

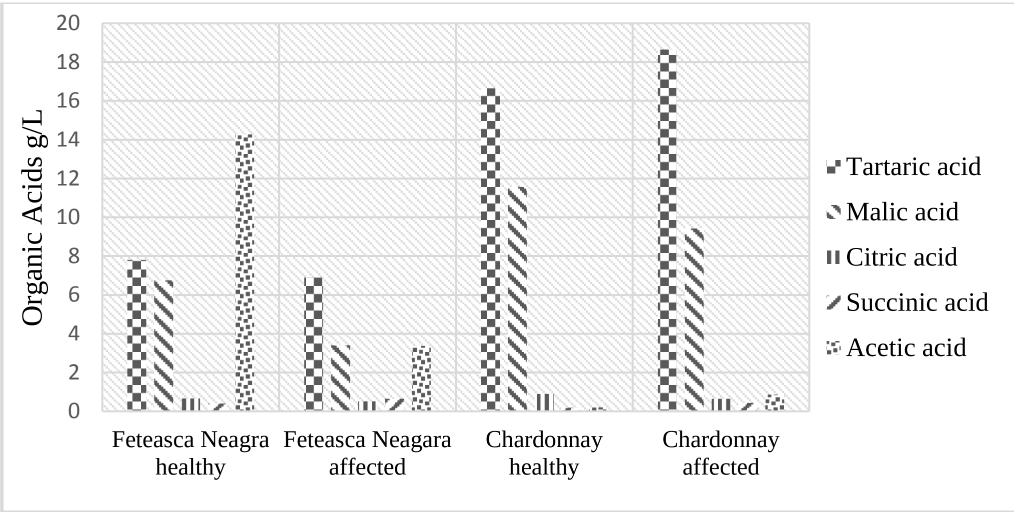

Figure 2. Organic acids concentration in Fetească Neagră and Chardonnay grapevine cultivars with and without phytoplasmoses symptoms

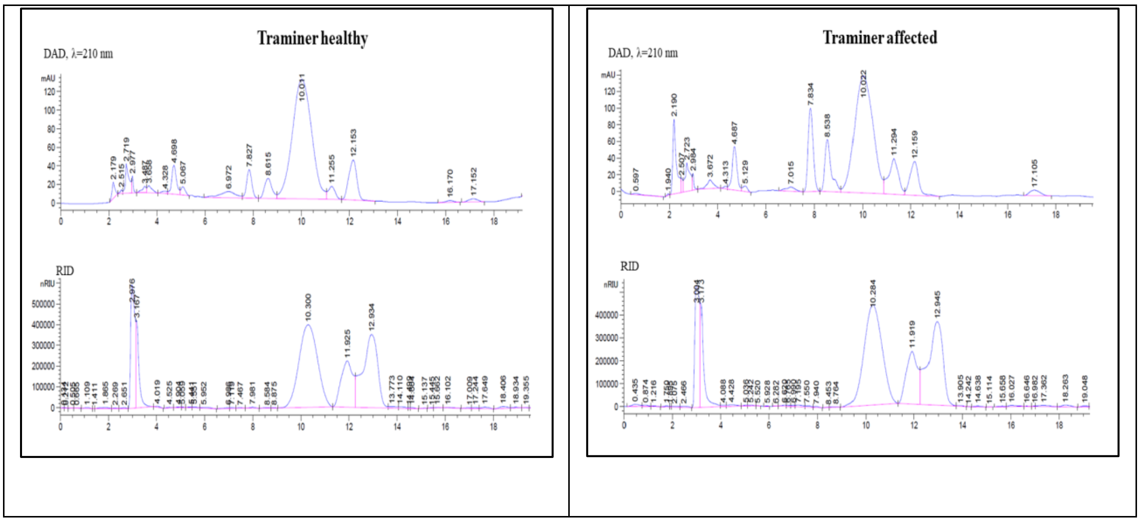

Figure 3. HPLC chromatograms of organic acids and sugars in the grape extracts of Traminer rose healthy and affected by phytoplasmoses

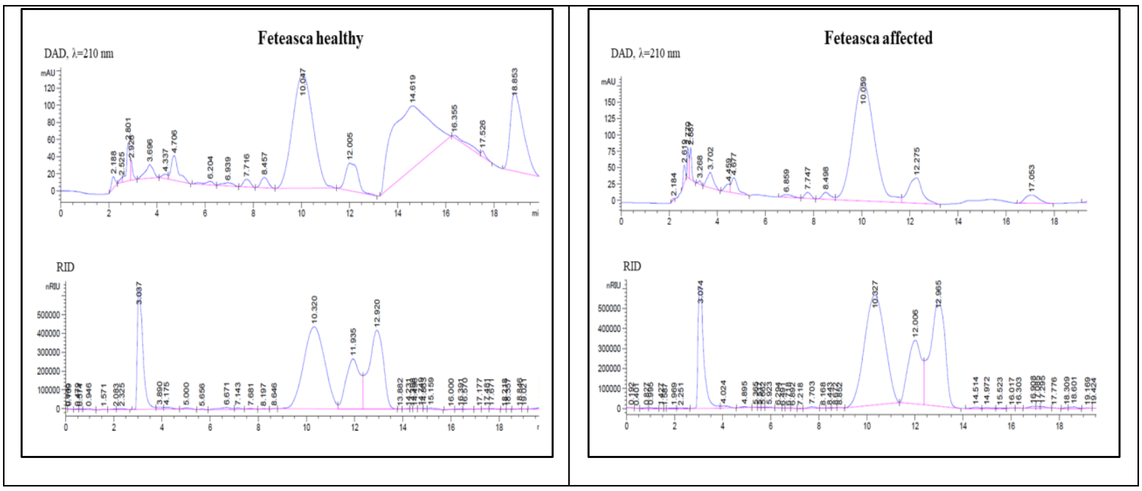

Figure 4. HPLC chromatograms of organic acids and sugars in the grape extracts of Feteasca Neagra healthy and affected by phytoplasmoses

Conclusions

◘ The results of this study showed that grapes from vines affected by phytoplasmoses symptoms presented higher content of total phenols and small quantity of total sugars and acids.

◘ Individual sugars glucose and fructose decreased quantitatively in all grape samples affected by yellows disease symptoms in both locations studied and that will influence alcoholic grades of wine. The value of glucose/fructose ratio remains constant for both affected and healthy samples.

◘ The concentration of flavanols is dominant compared to the concentration of phenolic acids in the affected samples, which can produce browning reactions in the winemaking process and affected wine quality.

◘ The amount of tartaric, malic, citric and acetic acids decreases in grapes affected than healthy grapes which will influence wine stabilization.

◘ This study allows to understand the direct impact of phytoplasmoses symptoms on grapes composition.

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