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ABSTRACT

of the PhD thesis with title:

THE INFLUENCE OF CULTIVATION TECHNIQUES OF BLACK WINE GRAPES AND OF THEIR PROCESSING ON THE RED WINE QUALITY AND PHENOLIC POTENTIAL"

Key words: phenolic compounds, quality, red wine, cluster thinning on vine, organic

The discussed theme in this thesis is part of an integrated internationally research regarding the development of wine industry by improving the quality of wines, that will meet the requirements of nowadays consumers for typical wines of terroir, rich in bioactive compounds, with beneficial effects on human health.

The research aims to set the principles to obtain red wines with a high phenolic content, from black grape varieties grown in the Murfatlar viticultural center, Murfatlar vineyard, by optimizing the cultivation techniques and grape processing.

The objectives through which was aimed to achieve the intended purpose are as follows:

1. The influence of cultivation techniques on the accumulation of phenolic compounds in black grape varieties from the viticultural center Murfatlar:

a) the cultural system - organic and conventional;

b) the application of a 30% cluster thinning on vine, compared to a control (without thinning);

2. Research to establish the optimal time of grapes phenolic maturity, raw material for winemaking:

- comparing two of the most used methods to measure the phenolic maturity of the grapes at harvest:

a) Glories

b) ITV France;

3. The influence of black grape processing technology on the degree of extractability and accumulation of phenolic compounds in the produced wines:

a) organic vinification;

4. The study of phenolic composition of the produced wines, by performing phenolic and anthocyanins profile of organic and conventional wines with and without additional cluster thinning on vine.

5. Performing correlations between phenolic parameters of grapes and produced wines in the experimental variants.

The PhD thesis is structured in two parts and contains 218 pages, which includes 34 tables and 94 figures and color photographs, the bibliography contains 200 references.

The first part of the paper refers to the state of the *art regarding the studied topic*, which includes the introduction and two chapters, and the second part represents the *personal contributions*, presented in four chapters, including the final conclusions and recommendations.

Chapter I, entitled ***Current status of research of the phenolic compounds from black grapes and red wines,*** presents the phenolic morphology and composition, the biosynthesis of phenolic compounds and the main factors influencing the accumulation of phenolic compounds from grapes and the phenolic compounds from grapes and wine.

In **chapter II** of this paper, ***Current status of research for the technology used for obtaining the red wines***, it is presented the classic technology used for producing red wines, the technology used for producing organic wines and the factors affecting the extraction of phenolic compounds during maceration.

Chapter III, *Research aims and objectives* makes the transition to the second part of the thesis, presenting the motivation and the purpose of research.

In **chapter IV**, ***Material and methods of research***, it is presented the area of origin of the biological material, the description of the selected varieties for the study, the applied cultivation technologies, the placement of experimental plots, vineyard characteristics and analytical methods used to achieve the objectives.

The biological material was derived from the experimental field of Research Centre for Viticulture and Enology Murfatlar, the physico-chemical analyses and structural characteristic determinations of the grapes and wines were conducted in the Laboratory of Grape Technologies and Chemistry of Wine, operating within the Murfatlar research station, and the determination of the individual phenolic compounds was conducted in the Laboratory of Chromatography from the Research Group of the Environment, Quality and Food Safety, of the National R&D Institute for Cryogenics and Isotopic Technologies – ICSI Ramnicu Valcea.

Were selected for the accomplishment of the determinations two vine varieties for red wines, Cabernet Sauvignon and Feteasca neagra, and two different cultivation systems, organic and conventional, with and without additional cluster thinning on vine, thus resulting 8 experimental variants:

- Feteasca neagra organic - without cluster thinning (Mt), V1
- Feteasca neagra organic - with cluster thinning (30%), V2
- Feteasca neagra conventional - without cluster thinning (Mt), V3

- Feteasca neagra conventional - with cluster thinning (30%), V4
- Cabernet Sauvignon organic- without cluster thinning (Mt), V5
- Cabernet Sauvignon- with cluster thinning (30%), V6
- Cabernet Sauvignon - without cluster thinning (Mt), V7
- Cabernet Sauvignon - with cluster thinning (30%), V8

To accomplish the objectives, research methods used included:

1. *Determinations regarding the time when the main phenological phases start (*budburst, flowering, veraison, harvest, leaf fall*).*
2. *Observations and measurements of the elements of fertility and productivity (*the relative and absolute fertility index, the relative and absolute productivity index*).*
3. *The usual methods for determining the quality of the grapes (*sugar content, total acidity*).*
4. *Spectrometric methods for determining the phenolic quality (*total anthocyans, extractable anthocyans, polyphenols, tannins from the skins, tannins from the seeds, seed maturity*).*
5. *Physical and structural determinations of grapes (*weight and number of clusters per vine, berries weight and rachis, the structure and composition index of the berry*).*
6. *The usual methods of analysis of wines (*alcohol concentration, total and volatile acidity, sugar content, total and unreducible extract, free and total sulfur dioxide, specific density*).*
7. *Spectrophotometric methods of analysis of wines (*anthocyans content by the pH variation method and total polyphenols, chromatic characteristics by Glories method and CIE Lab76 method, antioxidant activity by DPPH method*).*
8. *Chromatographic methods of analysis of wines for phenolic acids (*gallic acid, siringic acid, p-coumaric acid*), flavanols (*catechin and epicatechin*), flavonols (*rutin, quercitin*) stilbene, trans-resveratrol and mono-glycoside anthocyans (*malvidin, petunidin, delphinidin, peonidin and cyanidin*) acylated and coumarylated.*

Chapter V, ***Results and their interpretation***, represents the main part of the thesis, in which are presented the experimental results recorded for each variant, the statistical analysis and the interpretation for each treatment and variety, depending on the biological potential. The results are discussed for every year of study and for the three-year average (2012-2014).

The quality of grapes and especially their phenolic quality can be improved by applying the operations of cluster thinning on vine, both for organic and conventional system of cultivation. The physico-chemical and structural characteristics of the two varieties of grapes for red wines presented specific values for the variety potential, influenced by both organic and conventional systems and in particular by the cluster thinning operation.

The most important quantities of anthocyans were recorded in the case of organic system for the both variants for which the work of cluster thinning on vine has been applied, the increase value in the case of Feteasca neagra variety being 17.4% for organic system and 23.8% also with the thinning. For the Cabernet Sauvignon variety the increase in the determined value was 13% for organic growing and 23.1% for the cluster thinning variant. The extractable potential of anthocyans was significantly influenced by the cultivation technology, with the work of cluster thinning favoring the extractability percentage.

Grapes have a variable extraction potential, depending on the conditions during ripening and of the biological potential of the varieties. Cabernet Sauvignon, having a thicker and more resistant skin, had higher values than Feteasca neagra. The extractability notion of anthocyans depends on the ripeness state, which controls the decomposition of the skins, the lower the EA% value, the easier the extraction of anthocyans.

The extractability of anthocyans ranges generally between 70 and 20, but in the case of the analyzed variants it ranged from 45.7 to 60.4, small differences being observed for the biological potential of varieties. The organic cultivation system and in particular the work of cluster thinning facilitates the ripening process of the grapes and decreases the EA% values, although the differences were statistically insignificant. Tannic balance for a young red wine comes from the harmonization of the two classes of tannins; tannins that give the wine structure and body and the ones, from the skin, that gives fullness, roundness and color.

There is an increased risk of getting wines with excessive astringent if tannins from the seeds predominate, while bitter and herbaceous note is typical when too many tannins are extracted from the skins, especially if the grapes are not sufficiently ripen.

Tannin content from the skin had an average values between 22.6 to 30.6 mg/l for Feteasca neagra, and 33.2 to 37.5 mg/l for Cabernet Sauvignon, the cultivation technologies applied not significantly influencing this parameter. The content of tannins from the seeds presented similar values, ranging from 20.2 to 34.5 mg/l for both varieties.

Seed maturity index varies widely, taking values from 60 to 0, depending on the variety, the number of seeds per berry and the ripeness stage of the grapes. The the higher its value, the bigger the risk of a negative effect on the taste.

In the case of both varieties, regarding the phenolic quality of wines, the relevant variants are those in which the work of thinning was applied and those in which the organic cultivation systems was applied. The increase percentage of total phenolic compounds resulting from the application of cluster thinning operation, in the case of Cabernet Sauvignon variety it is 33.5% for organic system and 19.1% for conventional system. For anthocyans the accumulation percentage is higher by 23.8% for organic system and 23.2% for the conventional technology.

In the case of Feteasca neagra the increase percentage of total phenolic compounds due to the cluster thinning is on average 15.4% for organic system and 19.0% for the conventional technology, while in the case of the increase content of anthocyans this is 29.8% for the organic system and 25.5% for the conventional one.

The phenolic profile of the produced wines was unique, characteristic for each variety, the individual content in gallic acid, siringic, p-coumaric, catechin, epicatechin, quercitin, rutin, and trans-resveratrol, was influenced mainly by the work of cluster thinning and then by the cultivation system.

Anthocyanin profile, composed of individual quantities of simple mono-glycoside anthocyans (delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside, peonidin-3-glucoside, malvidin-3-glucoside), acylated (3-acetyl-peonidin glycoside, malvidin-3-acetyl-glucoside) and coumaryl (peonidin-3-glucoside coumaryl, malvidin-3-glucoside coumaryl) was characteristic for each variety, being also influenced by the two factors selected in this study. The anthocyan malvidin-3-glucoside, obtained the highest percentage in both varieties, the cluster thinning operation having a substantial contribution to its increase.

In the young wines deacylated free anthocyans predominated, confering the wine an intense color. These reached 83.72% from total anthocyans in the case of the organic system and 87.69% for the conventional cultivation system for Feteasca neagra, and 75.24% for the organic system and 80 63% for the conventional system for Cabernet Sauvignon. Differences between the two cultivation systems vary between 4.74 to 7.16% for both varieties.

The acylated anthocyans content (acetylated and coumarylated) increases in the case of cluster thinning on average for Feteasca neagra with 23.34% for the organic system and 18,45% for the conventional system and for Cabernet Sauvignon with 20.13% for organic and 28,41% for the conventional system, respectively.

When the cluster thinning is applied the antioxidant activity increases on average with 23.7% for Cabernet Sauvignon and with 18.59% for Feteasca neagra. An increase in the antioxidant activity is observed in the case of organic system compared to a conventional one, with values of 11.05% for Feteasca neagra and 5.97% for Cabernet Sauvignon.

With the increase of phenolic composition of wines, parameter a* presented medium and high positive values, corresponding to the coordinate red complementary color, confirming the participation of anthocyans as main components of the wine color. The values of parameter b* values were generally positive, the shades of yellow being preponderant rather than the blue ones.

As a general conclusion drawn from the analysis of experimental data, we can say that it was performed for the first time a phenolic and an anthocyan profile of wines obtained from

grapes grown organically and conventionally, whose potential quality, especially the phenolic one has been enhanced by the work of cluster thinning on vine, in the viticultural center of Murfatlar.

In the experimental variants carried on Feteasca neagra and Cabernet Sauvignon, there were identified different amounts of phenolic acids (gallic, siringic, p-coumaric) and flavanols (catechin and epicatechin), the quantities being significantly higher for the wines produced from grapes grown in the conventional system. The wines obtained from grapes grown organically presented a higher content in flavonols (quercetin and rutin) and trans-resveratrol. The highest content of trans-resveratrol was recorded for Feteasca neagra organic and Cabernet Sauvignon conventional.

Regarding the anthocyan profile it can be seen in both varieties a higher anthocyan content in the case of organic wines when compared with the conventional ones, with the exception of cyanidin-3 and peonidin mono-glycoside.

The antioxidant activity increased when the cluster thinning on vine was applied, on average with 23.7% for Cabernet Sauvignon and 18.59% for Feteasca neagra. An increase in the antioxidant activity it is shown for the organic system compared to the conventional one, with values of 11.05% for Feteasca neagra and 5.97% for Cabernet Sauvignon.

Red wine, by its complex composition, contains a number of antioxidant compounds such as phenolic acids, stilbenes (e.g. trans-resveratrol), flavonols (quercetin and rutin) and flavanols (catechin and epicatechin). These active compounds are beneficial for the human body by showing various protection functions, such as capturing free radicals, reducing oxidative stress and inflammation in case of atherosclerotic lesions, possible protection against cardiovascular disease, cancer, diabetes, Alzheimer's and some other neurological disorders. Though their bioavailability varies greatly depending on the class of polyphenols found in wine. Their metabolites, rather than the polyphenols by itself, could be the key compounds in the prevention of many diseases.

Based on the results of this study we can recommend adopting modern viticultural practices, such as cluster thinning on vine or organic culture, to improve the quality of grapes and in particular their phenolic potential, in order to produce the highest quality of grapes for wine, especially in the context of the increasing the demands of the consumers for foods rich in bioactive compounds with beneficial properties to health.

Due to the quantities of phenolic compounds identified in the wines produced for organic and conventional system, as well as the increase of these valuable sources through the cluster thinning operation on vine, we can produced wines with an improved functional potential. This

study showed that the best results were obtained in the case of Feteasca neagra organically grown and Cabernet Sauvignon conventionally grown.

Considering that wine is an important component in the diet, improving the phenolic potential and obtaining wines with sanogene properties leads to diversification of the range of wines, serving to encourage the adoption of a healthy lifestyle.