PhD THESIS

Quality conditions on wine grape black varieties for P.D.O. red wines classification in Transylvanian vineyards

(SUMMARY OF THE PhD THESIS)

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2021

INTRODUCTIONS

Wine authentication involves several aspects, such as geographical origin (PACKER, 2001), year of production, variety, producer and quality. It is important that the proof of the authenticity of the wine is based on those chemical parameters that do not change during vinification or that are difficult to falsify.

The P.D.O. certifies that the wine follows a route under constant control, from the plantation, to the technological process and to the sale. The P.D.O. guarantees that the wine in such a bottle is produced from a certain grape variety, grown in the declared area and that it is checked before bottling by an expert.

CURRENT STATE OF KNOWLEDGE

1. Cultivation of grape varieties for red wines

1.1. Cultivation of grape varieties for red wines worldwide. They are kept in medieval chronicles, registers of domains, monasteries or commercial houses, data on viticulture and wine trade, with phenological and economic observations on wines (COTEA et al., 2003).

1.2. Cultivation of grape varieties for red wines in Romania

The only grape variety for quality red wines that is found in the three stages (pre-phylloxeric, phylloxeric, post-phylloxeric) on the territory of our country is Fetească neagră. This variety resulted from the popular selection of the species Vitis silvestris Gmel. (OŞLOBEANU et al., 1991). Although the appearance in Romania of the Cabenet Sauvignon variety dates back to 1860, the national picture of the cultivation of red grapes for quality red wines began to be completed in the post-phylloxera period.

1.2.1. Assortment of noble varieties for red wines

Currently, from the assortment of noble varieties for wine grapes, a percentage of 74% is represented by white varieties, and the remaining 26% are varieties for red wines (ROTARU, 2009). In the 60's, wine growers in Romania reoriented and began to introduce the Merlot variety, from which in our country conditions can often produce wines of the same quality and production is almost doubled.

The phenomenon had a positive effect and the areas cultivated with the Merlot variety increase rapidly after 1965. The wine obtained from this variety being less harsh and can be consumed faster. It has penetrated many areas such as: Drăgăşani, Miniş, Recaş, the whole Dealu Mare vineyard, the Coteşti vineyard, the Râmnicului Sărat hills and the Galaţi vineyards (OŞLOBEANU et al., 1991).

The restoration after the phylloxera period of the Romanian viticulture brings in culture a new variety from France, namely Pinot noir. Having the same pretensions to the technological factors, it is found in the culture areas of Cabernet Sauvignon (OŞLOBEANU et al., 1991). From this variety are obtained the famous Burgundy wines. During the 70s and 80s, special wines were produced in the vineyards of Vâlcea and Mehedinţi, today the areas are restricted especially in the Dealu Mare vineyard.

2. Distribution of grape varieties for red wines

2.1. Worldwide spread – The spread of vine cultivation has spread across the globe in recent years.

2.2. Spread in Romania

In the South-East development region, the largest area under vines for D.O.C. wines were recorded. (44.5%), followed by the regions North - East (18.9%), South - Muntenia (11.0%), Center (11.0%), South - West Oltenia (9.3%). In 2009, the largest

areas cultivated with vines, for other wines, were registered in the regions: South - East (39.2%) and South - West Oltenia (20.5%).

3. The oenoclimate specific to grape varieties for obtaining wines in order to be included in the P.D.O.

If the surface on which the vines are cultivated is so large, the one on which quality wines are produced is considerably smaller (TEODORESCU et al., 1987).

4. The characteristics required of P.D.O.

OWN CONTRIBUTION

5. Motivation, purpose and objectives of the research

5.1. Research motivation. Graduating the TPVVSPD master's degree from the Faculty of Horticulture, USAMV Cluj-Napoca and then working since 2015 as a specialized inspector within the ONVPV directly involved in the control and authorization of D.O.C. and the certification of wines in this category, we aimed to study the conditions that favor not only the varieties for white wines, but especially those for red wines from northwestern Romania in order to be included in the P.D.O.

5.2. The aim of the research was to follow the quantity and quality indices of red wines from the varieties: Fetească neagră, Pinot noir, Merlot, from three locations in Transylvania, in terms of production and quality of wine grapes, from an oenological perspective, in in order to include these varieties in the requirements of the specifications in the PDO category.

5.3. Objectives pursued

Monitoring the development of ecoclimatic and ecopedological conditions where the research was carried out;
Variety production and quality;
Determinations on oenological indices of red wines;
Determination of total polyphenols;
Determination of flavonoid content;
Determination of antioxidant activity
Identification and quantification of phenolic compounds by subclass.

6. Material and methods

6.1. Biological material

6.2. Description of wines from experienced varieties

6.3. Organizing experiences: To study the influence of conditions on the quantity and quality of varieties, a bifactorial experiment was organized.

Factor A with 3 graduations, three varieties:
a1 - Merlot
a2 - Fetească Neagră
a3 - Pinot Noir
Factor B with 3 graduations, location:
b1 –Lechinţa-Bistriţa
b2 –Mica-Mureş
b3 –Camăr-Sălaj
The combination of experimental factors resulted in 9 variants.

6.4. Technology for obtaining red wines

6.5. Methods of work

6.5.1. Determination of alcohol concentration

The evolution of the vineyards cultivated with the three varieties from experience (for the location Lechinţa, Crama Liliac, DOC Lechinţa, is presented as follows: Merlot variety 1.80 ha, Fetească neagră 4.41 ha and Pinot Noir 5.70 ha. The surfaces remain constant in all three years of research.

8.2. Grape's production (q/ha)

For this purpose, the production of grapes will be done under well-specified conditions, referring to: - plantation density - minimum of 3,500 stems planted per hectare; Grape production is an important indicator by concretizing successive stages of fruiting, as a useful biological property (BUNEA, 2010).

8.2.1. Grape production obtained in experience for 2016

For the Lechinţa location, Crama Liliac, P.D.O. Merlot variety on an area of 1.80 ha we have a production of 215.00 q so 119.44 q / ha. For the Fetească neagră variety on an area of 4.41 ha we have a production of 528.5 q, so 119.84 q / ha. For the Pinot noir variety on an area of 5.70 ha we have a production of 683.9 q so 119.98 q / ha. For the Mica location, Villa Vinea Winery, P.D.O. Târnave of the Merlot variety on an area of 1.21 ha we have a production of 80.62 q so 66.62 q / ha. For the Fetească neagră variety on an area of 4.34 ha we have a production of 204.76 q, so 47.17 q / ha. For the Pinot noir variety on an area of 1.39 ha we have a production of 91.00 q so 65.46 q / ha. For the Camăr location, Fort Silvan Winery, D.O.C. Crişana for the Merlot variety on an area of 2.49 ha we have a production of 85.00 q so 34.13 q / ha. For the Fetească neagră variety, on an area of 1.46 ha, we have a production of 88.84 q, so 60.84 q / ha. For the Pinot noir variety on an area of 2.29 ha we have a production of 125.99 q so 55.01 q / ha.

8.2.2. Grape production obtained in experience for 2017

For the Lechinţa location, Crama Liliac, P.D.O. Merlot vinegar on an area of 1.80 ha we have a production of 214.00 q so 118.88 q / ha. For the Fetească neagra variety on an area of 4.41 ha, we have a production of 528.3 q, so 119.79 q / ha. For the Pinot noir variety on an area of 5.70 ha we have a production of 683.7 q so 119.94 q / ha. For the

Mica location, Villa Vinea Winery, P.D.O. Târnave of the Merlot variety on an area of 1.21 ha we have a production of 85.28 q so 70.47 q / ha. For the Fetească neagră variety on an area of 4.34 ha we have a production of 116.56 q, so 26.85 q / ha. For the Pinot Noir variety on an area of 31.46 ha we have a production of 1146.81 q so 36.45 q / ha. For the Camăr location, Fort Silvan Winery, P.D.O. Crişana for the Merlot variety on an area of 2.49 ha we have a production of 245.00 q so 98.39 q / ha. For the Fetească neagră variety on an area of 1.46 ha we have a production of 115.34 q, so 79.00 q / ha. For the Pinot noir variety on an area of 2.29 ha we have a production of 183.20 q so 80.00 q / ha.

8.2.3. Grape production obtained in experience for 2018

For the Lechinţa location, Crama Liliac, Lechinţa P.D.O. for Merlot on an area of 1.80 ha has a production of 215.89 q so 119.93 q / ha. For the Fetească neagra variety on an area of 4.41 ha we have a production of 528.90 q so 119.93 q / ha. For the Pinot noir variety on an area of 5.70 ha we have a production of 676.76 q so 118.72 q / ha. For the Mica location, Villa Vinea Winery, P.D.O. Târnave of the Merlot variety on an area of 2.20 ha we have a production of 159.55 q so 72.52 q / ha. For the black Fetească variety on an area of 5.84 ha we have a production of 383.90 q so 65.73 q / ha. For the Pinot noir variety on an area of 31.47 ha we have a production of 515.18 q so 16.37 q / ha. For the Camăr location, Fort Silvan Winery, P.D.O. Crişana for the Merlot variety on an area of 4.82 ha we have a production of 472.36 q so 98.00 q / ha. For the Fetească neagră variety on an area of 1.46 ha we have a production of 115.34 q, so 79.00 q / ha. For the Pinot noir variety on an area of 2.29 ha we have a production of 171.75 q so 75.00 q / ha.

8.3. Results regarding the oenological properties of the must

The acidity of the must was in values of 4.29% tartaric acid in the Camăr / Merlot variant, in 2018, being the lowest value and 6.51% tartaric acid in the Camăr / Pinot noir variants in 2016, respectively the Lechinţa / Merlot variant, in 2018. The maximum alcoholic strength of the wine is given by the sugar concentration of the must, it can reach the limit in which the yeasts can activate. The variant with the highest amount of sugar accumulated in the must is Lechinţa / Merlot in 2016 of 258.33g / l. respectively 254.19 in 2017. In 2018, the Camăr / Merlot variant 256.55g / l stands out. The amount of sugar with the highest values was accumulated at Lechinţa in all three varieties and in the other two locations the amount of sugar ensures the quality of the wine to be classified in the P.D.O.

8.4. Wine production results (hl/ha)

8.4.1. Wine production (hl) obtained in experience for 2016

The situation of wine production from the experience of 2016, exposed in the show that in the Lechinţa-Bistriţa area were registered the largest quantities of wine for all varieties compared to the two locations (Mureş-Mica and Camăr-Sălaj) in the 2016 experience year.

8.4.2. Wine production (hl) obtained in experience for 2017

The situation of the wine production obtained, presented in the show that in all three locations the production increased compared to 2016, with the exception of the Fetească negră / Mica variants (57.48 hl / ha).

8.4.3. Wine production (hl) obtained in experience for 2018

In the year of experience 2018 presented in it can be seen an increase in the amount of wine in the areas Small variety Pinot noir recorded low values compared to previous years.

8.5. Results regarding the oenological properties of wines

The total acidity is 4.80 g / l (tartaric acid) (Camăr / Fetească neagră) and 6.30 g / l (tartaric acid) (Mica / Merlot), in 2016.

In 2017 the situation is different, the Camăr / Pinot noir variant obtains the lowest value of 4.42 g / l (ac. Tartaric), and the highest is at the Camăr / Merlot variant of 5.70 g / l (ac. tartar).

The values for 2018 ranged between 4.10 g / l (ac. Tartaric) for the Camăr / Merlot variant, respectively 6.45 g / l (ac. Tartaric) for Lechinţa / Merlot, values close to those obtained by OŞLOBEANU et al. 1991, CAPRUCIU, 2011.

8.6. Results regarding total polyphenols according to the Folin Ciocâlteu method

Following the results obtained regarding the content in total polyphenols, it can be seen that the variant with the highest concentration is P7 (Merlot / Camăr, 2016, P.D.O. Crişana) of 0.321 mg / 100 ml, and the lowest P1 (Merlot / Mica, 2016, DOC Târnave) with the value of 0.173. The total polyphenol content of red varieties is higher compared to that of white varieties, due to the anthocyanins found in the skin (YANG et al., 2002), this fact confirms the similar situation for wine.

8.7. Results on total flavonoids

The values of flavonoids, presented in the nine samples of undiluted red wines show a higher concentration in sample P7 (Merlot / Camăr, 2016, PDO Crişana) with the value of 0.699 mg Q / ml, followed by sample 2 (Merlot / Lechinţa, 2016, PDO Lechinţa) with the value of 0.638 mg Q / ml on the last place was located the sample P9 (Pinot noir / Camăr 2016, PDO Crişana) with the value of 0.3635 mg Q / ml, the other samples fall in the middle zone.

8.8. Results regarding the antioxidant activity of mM Trolox wines (DPPH)

The antioxidant capacity in the case of wines from our experience shows that the Fetească neagră / Camăr variant, 2016, P.D.O. Crişana (P8) has the highest antioxidant capacity of 0.308 mM Trolox / l, followed by Fetească neagră / Lechinţa, 2016, P.D.O. Lechinţa (P3) 0.266 mM Trolox / l. The lowest value is recorded by the Merlot / Lechinţa variant, 2016, P.D.O. Lechinţa (P2) of 0.123ml Trolox / l.

8.9. Results on HPLC-DAD-ESI-MS analysis

8.9.1.1. The identification of phenolic compounds in wine was done using high performance liquid chromatography.

High performance chromatography was used to identify them. In table 8.26. it is possible to observe the identification of phenolic compounds by subclasses:

Stilben --- Resveratrol-glucoside (Piceid) and Resveratrol;

Hydroxybenzoic acid --- Gallic acid, Gallic acid stilester;

Hydroxycinnamic ac --- Caftaric acid (Caffeoyltartaric acid);

Flavan-3-oli --- Procyanidin dimer, Catechin, Epicatechin, Procyanidin dimer

8.9.1.2. Quantification. Analyzing individually (+) the catechin from the wine samples, the largest quantity is obtained for the Fetească neagră / Mica variant, 2016, P.D.O. Târnave (P4) of 22.167 mg / 100ml, and the lowest at P1 (Merlot / Mica, 2016, P.D.O. Târnave) of 9.572 mg / 100ml, (-) epicatechin obtains the highest value in the Merlot / Lechinţa variant, 2016, P.D.O. Lechinţa (P2) of 12.771 mg / ml, and the lowest Fetească neagră / Mica, 2016, P.D.O. Pills (P4) of 4.012 mg / 100ml.

8.9.2. Identification and quantification of phenolic compounds of the flavone subclass

It can be seen that the variant with the highest content in Kaempferol is registered at P5 (Pinot noir / Lechinţa, 2016 P.D.O. Lechinţa) of 13.755 mg / 100ml, with similar values are the variants from sample P3 (Fetească neagră / Lechinţa, 2016, P.D.O. Lechinţa) with the value of 11.618 mg / 100ml and P9 (Pinot noir / Camăr 2016, P.D.O. Crişana) with 11.402 mg / 100ml. Quercetin obtains the best value of 0.941 mg / 100ml, in the P4 sample (Fetească neagră / Mica, 2016, P.D.O. Târnave).

8.9.3. Identification and quantification of phenolic compounds of the anthocyanin subclass

In our case the variant with the highest value is Merlot / Camăr, 2016, P.D.O. Crişana (P7) with the value of 5,606 mg / 100ml, followed by P3, P2 and P1.

8.10. Results regarding the synthesis of the sensory analysis in order to classify the wines in the areas with Denomination of Controlled Origin (P.D.O.) from viticultural centers of Romania

Sensory analysis is an assessment based on the senses, and people, be they experts, have different degrees of sensitivity of the senses, they can get tired, the experience can be on certain wines, from certain areas, of a certain color or with certain flavors, intensity and the persistence of aromas in smell and taste may be a distinguishing factor between experts.

9. Conclusions and recommendations

9.1. Conclusions on the quality requirements, production and red wine, coloring substances and antioxidant effect of red wines from Transylvania in order to be included in the P.D.O. The notion of authenticity refers to something, come in this case, which is true, true, clean, unadulterated, original, which is in accordance with the truth, whose reality cannot be questioned.

Wine is not only a hydroalcoholic mixture, but also a complex product, rich in a number of constituents: sugars, alcohols, glycerols, organic acids, minerals and nitrates, polyphenols, vitamins and more than 500 aromatic compounds.

Through its products, viticulture is placed as a set of activities to achieve food production through the nutritional qualities of the wine product, but especially through the healing ones. Through moderate consumption, red wines are highlighted due to the prophylactic effect for a wide variety of actions.

The plantations from which P.D.O. it must have a varietal purity of at least 80%. The stems-impurities from these plantations will be part only of the varieties belonging to the species *Vitis vinifera*, and the grapes of these varieties will be separated at harvest from those of the variety that is the basis for the production of the wine for which the designation of origin is granted.

The meteorological data for each analyzed center show a global warming with positive influences for the cultivation of vines and red varieties. The thermal regime offers good conditions during the vegetation period, the temperature being over 19^0C which leads to obtaining quality grapes, implicitly of superior quality wines. Due to the humidity of the air and the annual amount of precipitation, values in excess of the minimum of 500 mm annually necessary for the culture are registered in all locations, which is beneficial 562 mm in Mureş, 701 mm in Bistriţa and 709 mm in Sălaj.

The evolution of vineyards cultivated with the three varieties from experience is as follows: Merlot 5.5 ha in 2016 and 2017 and 2018 a slight increase to 8.81 ha. Fetească

neagră 10.21 ha in 2016 and 2017 and 2018 a slight increase to 11.7 ha. Pinot noir 9.35 ha in 2016 and 39.45 ha in 2017 and 2018.
In 2016, the highest production of grapes per hectare is in the Lechinţa location for the Pinot noir variety 119.95q / h and the lowest values are obtained for the Camar / Merlot 34.11 q / ha variant. Regarding 2017, the highest production is obtained for the Lechinta / Pinot noir variant where there is a production is 119.93 q / ha and the lowest value is in the location Mica / Fetească neagră 26.85 q / ha. For 2018, the Lechinţa / Merlot variant returns to the forefront with a grape production of 119.93 q / ha, and the lowest value is at Mica / Pinot noir 16.38 q / ha.
The acidity of the must was in values of 4.29% tartaric acid in the Camăr / Merlot variant, in 2018, being the lowest value and 4.51% tartaric acid in the Camăr / Pinot noir variants in 2016, respectively Lechinţa / Merlot variant, in 2018.
The situation of the obtained wine production reveals the fact that the Lechinţa location in each year of study obtains in all varieties very close to the maximum limit imposed by the P.D.O. Lechinţa, respectively 78hl / ha. Small Location, P.D.O. Târnave obtains an annual average of 44 hl / ha for the Merlot variety, for the Fetească neagră variety an increase from 30.55 hl / ha in 2016 to 42.71 hl / ha in 2018 and for the Pinot noir variety it shows a decrease in production on hectare from 42hl / ha in 2016 to 8.26 hl / ha in 2018. The negative evolution of wine production per hectare is due to lower yields at the new areas that bear fruit in the third year and due to the sale of a quantity of Pinot grapes black. In the Chamber location, P.D.O. Crişana we record a constant increase in wine production per ha for the Fetească neagră variety from 36.30 hl / ha in 2016 to 51.33 hl / ha in 2018. For the Merlot variety in 2016 we have 19.15 hl / ha and in 2018 30.72 hl / ha. For the Pinot noir variety in 2016 we have a production of 7.06 hl / ha and in 2018 an increase to 48.73 hl / ha.
The very low production of Pinot noir wine in 2016 is explained by the sale of a quantity of grapes for vinification to third parties.
The maximum alcoholic strength of the wine is given by the sugar concentration of the must, it can reach the limit in which the yeasts can activate. The variant with the highest amount of sugar accumulated in the must is Lechinţa / Merlot in 2016 of 258.33g / l. respectively 254.19 in 2017. In 2018, the Camăr / Merlot 256.55g / l variant stands out.
The alcoholic strength acquired was between 11.70% vol. For the Camăr / Pinot noir variant and 14.98% vol. For the Lechinţa / Merlot variant, in 2016. For 2017, the lowest alcoholic strength was for the Mica / variant. Merlot 11.71% vol., On the first place being the Lechinţa / Merlot variant 14.75% vol. In the experimental year 2018, the first place belongs to the Camăr / Pinot noir variant with 14.78% vol.
The total acidity is 4.80% (tartaric acid) (Fetească neagră / Camăr) and 6.30% (tartaric acid) (Mica / Merlot), in 2016. In 2017 the situation is different, the Camăr / variant Pinot noir obtains the lowest value of 4.42% (tartaric needle), and the highest is in the Camăr / Merlot variant of 5.70% (tartaric needle). The values for 2018 were between 4.10% (tartaric needle) for the Camăr / Merlot variant, respectively 6.45% (tartaric needle) for Lechinţa / Merlot.
Wine is a living organism and therefore, any re-evaluation of a sample for inclusion in a designation of origin or geographical indication, can be done legally within a maximum of 90 days from the first tasting, provided that the control powders have been kept in favorable conditions, in order to avoid any kind of alteration. Even so, some experts

consider this interval to be too long, the quality of the wine being controlled by many more variables than the storage conditions.
Wines with a higher total concentration of polyphenols did not always show the highest values of antioxidant activity. The antioxidant activity of wines is more related to the type of individual phenolic compounds found in wines, than to the total phenolic content.
The polyphenolic profile of a particular variety largely reflects its genetic potential and can therefore be used as a tool to differentiate between different varieties. A type of soil favorable to the cultivation of vines, associated with favorable conditions of insolation, which benefits against the background of the management of the vegetative apparatus, ends with significant accumulations of dry matter in grapes and implicitly in corpulent wines, Premium quality.
This research is the first concrete step in demonstrating the favorability of the vineyards in Lechința, Târnave and Crișana, for the production of quality red wines, at least from the local Romanian variety Fetească neagră.
Following the spectrophotometric determinations performed on the nine samples of undiluted red wines, a higher concentration of flavonoids is shown in sample 7 Merlot P.D.O. Crișana. Also, sample 7 Merlot P.D.O. Crișana, shows the highest value of the limit for quantifying the anthocyanidin content. The highest polyphenol concentration was recorded in sample 7 Merlot P.D.O. Crișana, a result which, in conjunction with the study of flavonoids and antioxidants, shows by far the favor of obtaining red wines in Camăr, Sălaj. The accumulation of anthocyanins in grapes of black varieties is much increased at an average monthly temperature around 21°C for the Merlot variety, in P.D.O. Crișana and around 25°C, for the same variety, in P.D.O. Târnave.
The purpose of this study is to change the perception of consumers and to show winemakers and oenologists some concrete results, to show that in Romania, in today's climate change, it is not appropriate to have delimited areas only for white wines or only for those tomatoes. The highest amount of resveratrol is found in sample 3 Fetească neagră / Lechința, 2016, and regarding flavonoids and anthocyanins, the values are close to the Merlot / Camăr variant, 2016, P.D.O. Crișana.
Even if standardized descriptors are used, organoleptic assessment by sensory analysis remains an assessment based on the subjectivism of the senses with the constraint given by the experience and olfactory-gustatory memory of experts. The more expertise the experts used in the evaluation have, the less the subjectivism governed by the potential to appreciate the persistence and intensity of aromas, to position the typicality of a wine or its overall characterization.

9.2. Recommendation

It is recommended to authorized tasters to pay attention to the content of substances with antioxidant effect in wine when evaluating red wines from different cultivation areas.
With so many beneficial effects, wine consumed rationally, contributes to maintaining health and is recommended in the prophylaxis and treatment of diseases.

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