



ABSTRACT

Key words: ageing methods, antioxidant activity, aromatic compounds, color

Red wines maturation is considered a crucial stage of refinement, in which a series of physical, chemical and biological transformations take place, which improve wines stability and also modify the compositional and organoleptic characteristics.

Research related to this thesis was conducted in order to study the effects of different maturation processes, traditional (barrels) and accelerated/alternative (chips and staves) on volatile compounds and antioxidant capacity of Fetească neagră red wines.

The doctoral thesis contains 233 pages, and includes 12 tables, 62 figures and color photographs, 225 references and 6 attached documents, and is divided into two parts.

The first part of the work contains the introduction, the situation of wine and two chapters namely, the present state of knowledge regarding ageing techniques of red wines and red wines production technology by the classical method. The second part represents my own research, presented in five chapters, including conclusions.

In Chapter I, **PRESENT STATE OF KNOWLEDGE REGARDING AGEING TECHNIQUES OF RED WINES**, expose some generalities about the traditional maturation (oak wood barrels), alternative maturation methods (pieces of oak wood), wine composition modifications during maturation and evaluation methods of volatile compounds and antioxidant capacity for red wines.

In Chapter II, the **PRESENT STATE OF KNOWLEDGE ON TECHNOLOGY OF PRODUCTION RED WINES BY THE CLASSICAL METHOD**, briefly describe the classic technological process of producing red wines and the general technological flow.

In Chapter III, the **AIM AND OBJECTIVES OF THE RESEARCH** marks the passing to the second part of the thesis, **PERSONAL CONTRIBUTIONS**, presenting some considerations on the purpose and objectives of the research.

In Chapter IV, **ORGANISATIONAL AND INSTITUTIONAL FRAMEWORK OF RESEARCH ACTIVITIES**, are presented the research institutions where this study was conducted. Oenology Laboratory of the University of Agricultural Sciences and Veterinary Medicine "Ion Ionescu de la Brad" Iasi and Vitenol Laboratory from the University Cordoba, Spain are described.





In Chapter V contains the **RESEARCH MATERIAL AND ANALYSIS METHODS**.

In order to achieve this paper, we studied a local red grape variety, Fetească neagră harvested in autumn 2013, the vineyard Dealurile Fălciului, Șuletea.

The grapes from this vineyard were processed in the pilot station from the Oenology Laboratory of the Faculty of Horticulture U.Ș.A.M.V. Iasi respecting classical technology of winemaking. After stabilization, the wine obtained was divided in 19 experimental variants: each 5L of wine was placed in glass dishes to which were added 3 g/L and 5 g/L of oak wood fragments (chips and staves), and also 225 L of wine in three new barrels with three different toasting degrees (light, medium and medium plus). Thus, we obtained the following samples: V0 – control variant, V1 – stave (medium), V2 – stave (medium plus), V3 – stave (forte), V4 – chips (3 g/L, untoasted), V5 – chips (5 g/L, untoasted), V6 – chips (3 g/L, medium), V7 – chips (5 g/L, medium), V8 – chips (3 g/L, medium plus), V9 – chips (5 g/L, medium plus), V10 – chips (3 g/L, forte), V11 – chips (5 g/L, forte), V12 – chips (3 g/L, medium plus), V13 – chips (5 g/L, medium plus), V14 – chips (3 g/L, medium plus), V15 – chips (5 g/L, medium plus), V16 – barrel (light), V17 – barrel (medium), V18 – barrel (medium plus).

To determine the influence of various maturation processes on local wine from Fetească neagră were performed four analyzes groups:

- ❖ usually analyses methods: pH, total acidity, volatile acidity, alcoholic, reducing sugars, free SO₂, total SO₂ and relative density;
- ❖ special physico-chemical analysis: total phenolic compounds, total antioxidant activity, fractionation of phenolic compounds, color, identifying and quantifying the major and minor volatile compounds;
- ❖ sensory analysis;
- ❖ statistical analysis;

In Chapter VI are presented the **RESULTS AND DISCUSSION**, which comprises the biggest part of the thesis, containing the experimental results and statistical analyzes.

Regarding the maturation processes **influence on physico-chemical parameters** of Fetească neagră wines we can say that:

☼ pH of Fetească neagră wines increased during the maturation process in all the samples studied (staves, chips and barrels), except samples that were matured with american chips, where the values remained constant;

☼ alcoholic strength does not show significant variation between 1.5 and 3 months, where it appears that maturation processes do not influence this parameter. The highest values are





recorded in variants matured in barrels (15.49% vol.), compared with variants matured with staves (14.94% vol.) and variants with chips (14.96% vol.) at 1.5 months. The same trend is happening to wines aged for 3 months;

☼ total acidity decreases due to the precipitation of tartaric salts, esterification and other reactions that occur during maturation of wine, as well as due to the metabolism of malic acid and citric acid. The values are in the range of 4.45 g/L of tartaric acid and 6.00 g/L of tartaric acid at 1.5 months and 4.43 g/L of tartaric acid and 5.89 g/L of tartaric acid at 3 months. Examining the three maturation processes, we noticed that variants matured in barrels recorded the lowest values both at 1.5 months (4.45 g/L tartaric acid, 4.47 g/L tartaric acid, 4.52 g/L acid tartaric acid) and at 3 months (4.43 g/L of tartaric acid, 4.45 g/L of tartaric acid, respectively 4.49);

☼ volatile acidity is relatively small, the largest values being registered for wines aged in barrels both at 1.5 and at 3 months (0.71 g/L acetic acid and 0.84 g/L acetic acid). After three months, regardless of the maturation process used, the volatile acidity increases in all variants with 0.2–0.3 g/L. Volatile acidity increase is inevitable in these cases, not because of biological degradation, but due to the hydrolysis of acetyl group from hemicellulose wood, and due to the oxidation of some phenols from wine. This increase has not affected negatively the organoleptic quality of the wine;

☼ general trends observed in chemical parameters changes were similar for red wines matured using traditional methods (barrels) and alternative (staves and chips).

Based on gas chromatographic research was quantified for the first time **the volatile composition of Fetească neagră autochthonous wines** matured by various processes. In the same context, they have been calculated the odour activity value (OAV) for volatile compounds of wines studied. Thus, there were identified around 47 volatile compounds including alcohols, carbonyl compounds, aldehydes, carboxylic acids, esters, lactones, terpenes, volatile phenols, compounds of the oak wood. It was also found a large number of flavor compounds for both traditional maturation of Fetească neagră wines (45) and for the alternative maturation (47), which reveals similar characteristics regardless of the aromatic profile of the maturation process used.

In the present experiment, using the traditional maturation method (barrels) and two alternative methods (staves and chips) were identified seven aromatic compounds (key) from three different chemical classes: furan compounds (furfural, 5-methylfurfural and





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5-hydroxymethylfurfural), volatile phenols (guaiacol and 4-vinylguaiacol) and wood compounds (*trans*-whiskey lactone and *cis*-whiskey lactone).

The concentration of furfural increased after three months maturation for variants that used staves, chips, barrel and the control sample. Thus, for samples matured three months, with the intensification of the toasting degree we observe an increase of furfural concentration.

For 5-methylfurfural we can observe that the highest concentrations are recorded for variants matured with staves, namely 1400 µg/L. Regarding samples with chips and in barrels, we can mention that the values are similar regardless of the dose used and the degree of toasting. It can be seen that the variants that used untoasted chips present small amounts of 5-methylfurfural.

5-hydroxymethylfurfural compound was identified only in variants aged for three months in barrels. It presents concentrations of 92 µg/L for the variant with medium toasting degree and 125 µg/L for the variant with medium plus toasting.

Fetească neagră wine matured in barrels shows small amounts of guaiacol, while wines samples matured with staves and chips presents much higher quantities. As can be seen, the concentrations of guaiacol and 4-vinylguaiacol in all variants aged three months increased, except samples that used medium toasted chips (5 g/L), where the concentration decreased slightly.

Generally, it can be considered that *cis* and *trans* isomers of β-methyl-γ-octalactone (oak wood lactone or whiskylactone) are largely responsible for the matured character in wines, mainly for reminiscent notes of coconut, vanilla and toasted wood.

In the control sample, *trans*-whiskey lactone was not identified but otherwise, in all other maturation processes this compound was quantified and the concentrations increased with the duration. *Cis*-whiskey lactone content was not identified and quantified in variations with untoasted chips. Concentrations are higher for variants at three months than 1.5 months, and are generally much higher than the *trans*-whiskey lactone.

Multivariate analysis of variance (MANOVA) was performed in order to determine which factors had a statistically significant effect on concentrations of volatile compounds of matured wine samples and to assess the importance of interactions between factors. It clearly showed very significant statistically influence exerted by the tested factors (maturation duration-T, toasting degree-GP and dose-D, for chips samples).

Alcohols are quantitatively the group with the highest concentration of volatile compounds in Fetească neagră red wines. Content of total majority alcohols was significantly





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dependent on the degree of toasting and time for wine matured with barrel or staves and dependent on the duration of maturation and dosage for samples with chips. Content of total minority alcohols was identified as dependent on the time factor for wines matured alternative and on time and the toasting degree factors for wines matured traditional. As to the amount of majority and minority alcohols, we see a slight increase after 3 months of aging.

Carbonyl compounds identified in all maturation processes, acetaldehyde and acetoin are significantly dependent on the three factors and the interaction between them. There is a concentration increase after three months for acetaldehyde and acetoin in all experimental variants.

Total aldehydes identified in Fetească neagră wines matured with staves, chips and barrel were dependent to all three factors (T, GP and D). Similar to wines matured with staves chips or barrel, furfural and 5-methylfurfural show the highest concentrations after three months. Only in wines aged in barrel after three months was identified another important furan namely 5-hydroxymethylfurfural that has a strong almond flavor.

The total carboxylic acids content was found to be dependent on both the duration of maturation and the degree of toasting and also their interaction for wines matured with staves chips, while for wines matured in barrels the time and toasting factors are insignificant.

All majority esters identified in experimental variants showed a significant dependence of all factors (T, GP, D). While minority esters identified variants matured with chips were not dependent factor D and minority esters present in variants matured with staves or in barrels were dependent on T and GP.

Following GC-MS analysis and experimental data processing were identified the most important lactones of red wines, four of which (γ -crotonolactone, γ -nonalactone, γ -decalactone, γ -butyrolactone) are above the perception threshold and, given fragrances descriptors (coconut, peach, caramel, toast), positively influencing the overall flavor of wines. Total lactones identified in all maturation samples have a significant dependence of the three factors (T, GP, D).

Among terpene compounds found in wines aged with staves, chips and barrel, only limonene was noted, whose content was significantly dependent on the degree of toasting and time for wines matured in barrel or with staves and dependent only on the time factor for wines with chips.

In the analyzed wines samples, were identified guaiacol and 4-vinylguaiacol as volatile phenols and the highest concentration was found for 4-vinylguaiacol in wines aged three months.





Total volatile phenols content was significantly dependent of time, the toasting degree, the dose used and the interaction between factors.

Of the wood compounds, *trans*-whiskey lactone and *cis*-whiskey lactone in wines aged with staves or barrel are significantly dependent on time and toasting degree and wines that used chips are only dependent on the toasting degree, therefore time and dose of chips does not influence the concentration of these specific compounds.

Two **discriminant analyses** have been made to determine the differences between the wines due to maturation processes. For the classification of wines produced were used in the first study values of majority and minority volatile compounds, and in the second values of antioxidant activity and their phenolic fractions. In both studies there is a clear differentiation of all maturation processes, forming four groups that include the control samples, variants with alternative processes (staves and chips) and variant matured by the traditional method (barrel).

A **cluster analysis** was performed, and to classify the obtained variables were used volatile compounds values. Following this statistical analysis, both at 1.5 and 3 months have formed two groups: the first contains wines with staves, chips and control sample, while the second group includes only wines matured in barrels.

The aromatic profile of Fetească neagră wines included chemical, fruit, velvety, butter, floral, vegetable, roasted, spice and woodseries. Wines matured through alternative maturation processes (staves and chips) have similar OAV, while wines matured traditional differs from the alternative ones throw smaller chemical, fruit and fatty acids series and larger citrus, wood, toasted and spice series.

The greatest **odours activity value** was obtained by wine aged with american chips (OAV – 558 at 1.5 months and OAV – 684 at 3 months), indicating that this is the wine with the most complex flavor. Wine matured with stave have OAV equal to 256 at 1.5 months and 354 at 3 months and for wines in barriques, a OAV of 108 at 1.5 months and 136 at 3 months.

We can conclude that as demonstrated by the values for the odor activity and sensory analysis, the wines matured by various processes (stave, chips and barrel) are characterized by intensity of fruit flavors that gradually subsides during aging and specific matured wines notes, like of wood, smoke, spice, cocoa and vanilla.

So far there is no published research on **antioxidant capacity** and **phenolic fractions** for red wines matured through traditional and alternative methods and so, this paper brings new information on their antioxidant potential *in vitro*.





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Wine is a dynamic system, constantly evolving, where numerous reactions involve the polymerization and condensation of phenolic compounds during the maturation process. These reactions affect undoubtedly their structure and antioxidant effect. Results of the antioxidant capacity were expressed as mM Trolox and were determined by the ABTS^{*}, repeating three times the samples studied.

Of the three maturation processes, the highest content of phenolic compounds for unfractionated samples is recorded in wines matured with staves for three months.

The total antioxidant activity of unfractionated samples decreased for control samples matured three months, however, was constant in wines matured with medium plus chips and increased in all other variants. From all maturation processes, the highest values recorded for total antioxidant activity are in variants that used medium and medium plus barrels.

Phenolic composition of oak wood includes phenolic acids, flavones, coumarin, gallic and ellagic tannin, among other compounds. These compounds are soluble in hydro-alcoholic medium and are released gradually during the aging process of the wine, thus changing the composition of the phenolic fractions.

Phenolic fractions are represented by phenolic acids (fraction 1) and flavanols (fraction 2) that decrease according to time for wines matured with chips and increase for samples matured with staves and barrels, while flavonols (fraction 3), anthocyanins and procyanidins (fraction 4), having high molecular weight increase in all types of maturation.

At 3 months, total antioxidant activity (TAA) of the fraction 1 decreased during maturation in variants with staves or chips, while values increased in barrel matured samples. Fraction 2 shows increased antioxidant activity in all experimental variants compared wines matured with 1.5 months, except variants with low levels of toasting. For fractions 3 and 4, TAA increased with the duration of the process in both, control variant and all other three methods.

A high content of phenolic compounds does not involve automatically a high antioxidant activity, because it is influenced notably by their chemical structure and not by their total concentration.

According to the general trend, the ratio between TAA and phenolic compounds decreases with maturation duration and with the intensity of staves toasting degree and increases with dose and maturation duration of chips and with the maturation duration of barrels.

Regarding total phenolic compounds, the MANOVA analysis of wines matured with staves points out that they have been dependent on two factors (time and toasting degree) and the interaction between them, and wines matured with chips were dependent of time, the toasting





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degree and not to the used dose, and finally wine aged in barrels or staves were dependent on both these factors.

Regarding TAA, MANOVA analysis shows that wines matured with staves was not dependent on the time and not the toasting degree, but to the interaction of two factors, and that samples with chips were not dependent on the dose used, but the maturation time and the toasting degree and finally wines in barrel were dependent only on the time.

These results confirmed that Fetească neagră tested wines are a good source of antioxidants and therefore a moderate drinking habit can have a beneficial effect on human health.

Chromatic characteristics are influenced by the maturation process used. The wines aged with 5 g/L of chips with a medium plus and heavy toasting level were more brownish than the same wines aged with 3 g/L of chips. Examining the three maturation processes, wines matured in barrels had a less saturated color than wines aged with staves and chips. All wine samples have low lightness, which is linked to the maceration process during fermentation and to the formation of color pigments during maturation. The most intensely colored wines were obtained by maturation with low toasted chips, and most tinted were obtained for barrel maturation.

Depending on the parameters L^* , a^* and b^* there is a similarity between variants of wine matured with staves, chips and control sample. And so, we can distinguish wines matured through the two alternative processes from samples matured by traditional methods.

