ABSTRACT

This thesis aims to obtain data on the qualitative and quantitative characteristics of phenolic compounds, especially tannins of white and black grape varieties from Iaşi vineyard, during their maturation, as well as data regarding wines made by different technological processes, and data regarding the exhausted material (skins and seeds) resulting from the winemaking process.

All these aspects have been studied during two years, in 2010 and 2011, to analyse the influence of the climatic conditions on some compositional characteristics of the grapes and wines produced.

The doctoral thesis has 239 pages, of which 33 tables 86 figures and color photographs, bibliography containing 152 titles and 24 annexes. It is structured in two parts. The first part contains the introduction and the first two chapters regarding the current state of knowledge of the issues addressed, and the second part represents personal researches presented in five chapters including conclusions.

Chapter I, entitled ***Current state of research on the phenolic compounds of grapes and wines*** is divided into two sections. In section 1.1, the general concepts on phenolic compounds in grapes and wine are presented, special importance being given to their importance and classification.

Phenolic compounds play an important role in wines, being involved in defining its organoleptic characteristics, intervening on firmness, roundness, astringency, hardness, smoothness and even playing a role in the composition of their typicality (Popa A., 2008).

Given the presence of other groups in the molecule(–H, –O–CH_3, –COOH) to the group –OH, as well as some residues of other substances (sugars, acids etc.), in the category of phenolic compounds a certain group of compounds with phenolic character given by phenolic acids (hydroxybenzoic, hydroxycinnamic, stilbenes), volatile phenols, tannins (hydrolysable and condensed), color substances (anthocyanidins and flavones) (Garrido J. and Fernanda Borges, 2011) can be included.

In section 1.2 data on the properties of tannins in grapes and wines and tannins classification are presented.

Tannins are found in all organs of vines including grape bunches respectively, skins, seeds and pulp. Up to 20% of the amount present in grapes, can pass into the must and/or wine, according to the applied technology.

Depending on the structure and properties, such substances are divided into hydrolyzable tannins (galotannins, elagotannins) and non-hydrolyzable (catechins, proanthocyanidins).

In Chapter II of this thesis, ***The current status of research on the technology of red and white wines*** is presented. The white wine technology of production is the most demanding because grapes must be made within a short time and must contact with air and solid parts of the grapeshould be avoided, if possible. Mechanical interventions on the grapes must be soft, as to avoid crushing the solid parts (clusters, seeds, husks) and must oxidation. Any techological defect, however small, is reflected in the quality of white wines, especially in color and taste (Ţârdea C., 2007).

There are two trends in the technology for producing white wines:

- Obtaining varietal wines;
- Obtaining technological wines.

Obtaining quality red wines is influenced by how the wine-maker can harmonize the peculiarities of the vineyard and those of the grape varieties with the winemaking techniques, as well as the stabilization and fining of wines, resulting in a wine with a distinct identity (Cotea V.V. and Cotea V.D., 2006).

Variants adapted and used in this study are:

- Production of red wines by the classical method
- Production of red wines in rotative tanks;
- Production of red wines by thermomaceration;
- Producing red wines by microwave maceration.

In Chapter III, ***The aim and objectives of the research*** are presented, making the transition to the second part of the thesis ***personal contributions*** presenting some considerations on the purpose of research, complemented by activities undertaken under each track.

In Chapter IV, ***Organisatoric and institutional frame of the research activities***, the institutions where this research was conducted are presented. The Oenology Laboratory is from the farm "V. Adamachi "and Enology Research Center of the Romanian Academy, Iasi

Branch. Also, the Oenology Laboratories within the Wine Institute in Athens and the Agricultural University of Athen sare presented.

Chapter V presents ***The research material and analysis methods***. This chapter includes data on: the origin area of used vine varieties; description of these varieties and how to obtain extracts from skins and seeds; adapting technological processes for producing wines in experimental conditions; analytical methods used.

To achieve the objectives of the research, the following grape varieties were analyzed, harvested from Iaşi vineyard, as well as the wines produced from them through various winemaking technologies:

- In 2010, grapes of Chardonnay, Sauvignon Blanc and Cabernet Sauvignon were harvested from SCDVV Iaşi; Fetească albă, Fetească regală, Fetească neagră and Merlot, harvested from the didactic farm "V. Adamachi " and lastly, Băbească neagră harvested from Cetăţuia Hill – Cetăţuia Monastery.
- In 2011, Chardonnay and Sauvignon blanc grapes were harvested from SCDVV Iaşi; Fetească albă, Fetească regală and Merlot harvested from the didactic farm "V. Adamachi ", Băbească neagră harvested from Cetăţuia Hill – Cetăţuia Monastery; Fetească neagră and Cabernet Sauvignon harvested from Cozmeşti farm (VINIA SA).

Analyses were made using accredited methods indicated in national and international standards, as well as in specific literature:

- Common methods of analysis: analysing of dry substance, analysing the alcoholic strength, analyzing total acidity and volatile acidity, pH, analyzing reductive sugars, analyzing total dry extract as well as non-reductive extract, analyzing free and total SO2 content, analysing conductivity, relative density and permanganate index (IMN).
- Analytical methods using spectrophotometric techniques: analysis of polyphenols (IPT) or D280 index, Folin-Ciocalteu index (Fc), anthocyanins, analysis of total tannins (methylcellulose index), analysis of astringency, of procyanidins, of HCl (degree of polymerization of procyanidins in wine), analysing the color of wine by means of the CIE L * a * b * 76.
- Methods of analysis that use the HPLC technique (High-Performance Liquid Chromatography): identification of phenolic acids, shikimic acid, resveratrol; separation and identification of anthocyanins; identifying tannins.

Chapter VI ***Rezults and discussions*** includes the largest part of the thesis, the experimental results being presented and finally a statistical analysis and interpretation.

Regarding the characterization of grapes during the maturation period in 2010, the analysis of data on the grapes phenolic content (ie, the skin and seeds) says the following:

- The degree of ripening of the grapes influences the content of phenolic compounds, in the sense that an advanced degree of maturation is equivalent to a higher quantity of phenolics;

- On the content of total tannins in grapes (skins and seeds), it was observed that the degree of maturation of the grapes affect the total tannin content, meaning that a more advanced degree of maturation is equivalent to a decrease in the amount of tannins.

Regarding **the characterization of 2010 wines**, the values of phenolic acids in Chardonnay wine, prefermentative sample, which has the highest values concerning almost all identified phenolic acids. The differences between the values obtained from white wines are generated especially by the variety factor, and less by the technological factor.

In red wines, it can be seen that the values of shikimic acid content is higher in the case of experimetal samples, especially when heat was used, in particular thermomaceration, but also in the case of microwave maceration.

It is noted that, in the case of white wines, the pre fermentative sample, the highest values regarding the resveratrol content are registered compared to the values obtained using the classical technology, and this is due to longer periods of contact of the must with the marc, which leads to a better extraction of the compound from the skins of grapes. One can not say that the technological factor influenced the high resveratrol content of red wines, it was only the grape variety and its potential for accumulation of such compounds.

The analysis of total phenolic compounds content in white wines is regarded in terms of technology. The pre-fermentative sample has values higher than the classic version because there was a longer contact with the grape marc, and a better extraction of of these polyphenolic compounds from grape skins and seeds was thus possible. It seems that the prefermentative wine sample provides a higher phenolic content than wine obtained by the classical technology. Analysing the total phenolic content after one year of aging white wines, it is observed that it decreased in all experimental variants, most likely after polycondensation processes occurring in wine.

From the technological point of view it is noted that red wines produced by technological variants with use of heat (thermo-maceration, maceration using microwaves), showed the highest values of total phenolic compounds. A good extraction of these compounds from grapes can be achieved using these technological variants.

The highest values of anthocyanin content were recorded in all microwave maceration wine samples. The explanation could be that the heat treatment, in this case, microwave maceration, influences the extraction of these compounds. After a year of maturation, the anthocyanin values decreased in most samples, regardless of variety or technology. This decrease in anthocyanin content is explained by the fact that they condense – by forming flobafenes that precipitate (Cotea V.D., 1985).

Comparing the values of total tannin content of white wines, one observes that, technologically, the prefermentative sample has higher values than those obtained by the classical technology. This is explained by the fact that the must stayed longer in contact with the marc and thus the extraction process of the tannins from the skin and seed were favored.

The Băbească neagră wines have values of the total tannin content lower than the values obtained for white wines, and this can be explained by a variety determinism or through the bad health shape of the harvested grapes or by harvesting at the wrong time, things that could not be controlled experimentally.

Regarding the content of procyanidins, both minimum and maximum values of these compounds were registered, regardless of the technology used for wine-making.

The white wines obtained by the classical values have higher degree of polymerization, so pre fermentative process seems to stimulate depolymerization of these compounds. In red wines, it can be observed that HCl index has values that are higher in technological variants implied use of heat (maceration using microwaves and thermo maceration) during the maceration-fermentation.

Analyzing the astringency values of white wines, one can note that what makes the difference between these results is the technology of winemaking. In all variants before fermentation lower values are observed, compared to the values of classical variations, this is explained by depolymerization action of astringent compounds that give exerted by technological solution before fermentation. The obtained values have a varietal influence on the content of compounds that give astringency to red wines. Wines obtained from Cabernet Sauvignon and Fetească neagră have higher astringency values than the wines obtained from Merlot and Băbească neagră.

Higher values of antiradical capacity of wines made from indigenous varieties Fetească Regală and Fetească albă compared to the cosmopolitan varieties Chardonnay and Sauvignon blanc were observed. The longer the antiradical capacity conferred an increased amount of phenolic nature antiradical. We note that these higher values prevail and red wines

obtained by macerating technology variants - classic fermentation and maceration - fermentation in rotating tanks.

Regarding **the exhausted material** resulting from the process of winemaking in 2011, a number of issues are mentioned below.

From the technological point of view, it is observed that the phenolic content values in exhausted material (skins) obtained from the prefermentative maceration of white wines is lower than the values of the classical variant. Black grape varieties, except Fetească neagră, showed a higher value than the samples obtained through classic technology; it seems that the technological options which involves the use of heat (thermo maceration and maceration using microwaves) had higher values of phenolic content values influenced by the fact that there was a lower extraction of these phenolic compounds in the skins during maceration-fermentation process.

Also in terms of technology is observed that the values phenolic content of exhausted material consisting of seeds, resulting in the pre fermentative sample are lower than the values of classical variant. It is noted that the conventional technology influenced to a large extent the content of phenolic compounds in the exhausted material (seeds) after obtaining red wine. With the exception of Cabernet Sauvignon which has a higher value at the variant microwaves maceration technology, all the other values are higher in the classical sample.

In terms of technology, it is observed that the values of total tannin content from the exhausted material, made of grape seeds, resulted from the prefermentative sample, had smaller values than the classic variant values. Indigenous varieties Fetească regală and Fetească albă had much higher values than cosmopolitan Sauvignon Blanc and Chardonnay varieties, so their seeds remained with higher amounts of tannins in their composition probably due to their specific tissue characteristics.

Similarly, in terms of technology, it is noted that the total tannin content values from the exhausted material consisting of grape seeds, resulting in the version before fermentation to obtain white wines are lower than the values of the classical variant. Sauvignon blanc and Chardonnay varieties values of total tannin content are much higher than the values obtained from local varieties and Fetească albă and Fetească regală, so their seeds are left with large amounts of tannins in composition. The data obtained can not be said that technological alternatives methylcellulose index influenced values of depleted material consisting of seed from red wines because there have been both minimum and maximum values regardless of the technology, but perhaps histological peculiarities variety

Profile analysis of tannins in 2011 white wines shows that:

Catechin - is present in all wines analyzed before fermentation, the values are smaller in the prefermentative version than in the classic version, this being explained by the fact that an enzymatic degradation of catechin before fermentation would be facilitated.

Epicatechin – the values obtained in the prefermentative variant are higher only in wines from Chardonnay and Sauvignon blanc varieties, while in wines from indigenous varieties Fetească regală and Fetească albă, the values are higher in the classical samples.

B_1 dimer – the values obtained in the prefermentative variant are lower in wines from Chardonnay and Sauvignon blanc varieties. In wines from indigenous varieties Fetească regală and Fetească albă, the values are lower in the classical samples.

B_2 dimer – wasn't identified in Fetească regală and Chardonnay wines; in Sauvignon blanc wines it was identified only in the prefermentative sample.

C_1 trimer wasn't identified in Fetească regală, Fetească albă and Sauvignon blanc wines.

EGCG (epigalocatechin-3-orto-galat) was identified only in the prefermentative sample of Fetească regală wines.

CG (catechine-galat) was identified only in the prefermentative sample of Chardonnay wines.

EGC (epigalocatechine) wasn't identified in Sauvignon blanc wines.

ECG (epicatechine-3-orto-galat) wasn't identified in any wines.

Tannin profile analysis of 2011 red wines underlines the fact that the values of the following identified compounds catechin, B_1 dimer, B_2 dimer, C_1 trimer, CG (catechin-galat), EGC (epigalocatechin) ECG (epicatechin-3-orto-galat), are not influenced by the technological processes, as both minimal and maximal values have been registered no matter the technology used.

The highest values of epicatechine and EGCG (epigalocatechin-3-orto-galat) are found in samples where heat was used(thermo maceration, microwave maceration).

Chapter VII presents ***The conclusions***.

Variability can be found regarding the evolution of total phenolic compounds' accumulation in all studied varieties, as well as the moment of reaching their full phenolic maturity.

The white varieties have in their seeds a higher quantity of total tannins than in the seeds of black varieties, an inverse ration being registered in the skins.

The positive influence of the prefermentative technology was strongly seen in the case of total phenolic compounds and total tannins in white wines from both harvest years.

Alongside the black grapes variety's specificity regarding their content of total phenolic acids and total tannins, the influence of more extractive technologies was also registered (thermo maceration and microwave maceration), generating higher values of these compounds.

There are differences regarding the values of total phenolic compounds and total tannins from exhausted material (skins and seeds) remained after wine making, differences induced by the used technology (extractive technologies lead to low remnant values) as well as the grape variety factor, which determines the accumulation capacity.

By applying statistical tests, a very significant statistical influence was found to be connected with the three analysed factors (harvest year, technology and the synergy of the two) on the total tannin content and the identified tannin profile.

A stringent need for continuation of the research regarding tannin structure in grapes, wine and exhausted material is in order. Analyses should be performed even before the wine-making process begins.