SUMMARY

Key words: *polyphenolic fingerprinting, LC-MS, red wines, total polyphenol content, antioxidant activity, statistical modeling, principal components analysis, authenticity markers*.

The doctoral thesis entitled ***„NEW CONTRIBUTIONS REGARDING THE FINGERPRINTING OF THE POLYPHENOLIC PROFILE OF DRY WNES FROM FOREIGN AND AUTOCHTHONOUS RED VARIETIES”,*** elaborated by the PhD student Laurențiu Mihai Palade, under the supervision of Prof. Univ. Dr. Mona Elena Popa, within the Doctoral School of the University of Agronomic Sciences and Veterinary Medicine, Bucharest, evaluated the issue of polyphenolic fingerprinting for red wines from recognized viticultural areas on the territory of Romania.

The aim of the thesis was the investigation of the polyphenolic potential which contributes to the quality of red wines, as well as establishing a significant base frame for further research.

Under these conditions, there were detached some important objectives, as follows:

- Evaluation of the polyphenolic content and the antioxidant potential of the grapes and of the resulted wines, respectively, originated from red varieties from the country;
- Study of the relationships formed between the polyphenolic compounds and the influence factors;
- Detailed analysis of the interactions between the identified polyphenols by use of internationally recognized statistical methods;
- Identification of the elements associated to the polyphenolic fingerprinting with important implications on the authenticity and typicity of the variety and area of origin of red winesi.

The paper is structured in three parts which are comprised of 14 chapters with a rich content of descriptive elements (figures, schemes, tables).

The first part of the thesis presents general aspects that envisage the current state of knowledge regarding the polyphenolic diversity that is characteristic to the red grapes and the resulting wines.

Chapter I, entitled **„Characterization of the polyphenolic compounds in the grape constitutive elements (skins, pulp, seeds, juice) and in the red wines, contributing to the**

fingerprint of authenticity and typicity of variety and area of origin" represents a literature review and analyses the general aspects regarding the synthesis, the incidence and the evolution of the polyphenolic compounds in red grapes and wines.

In the second chapter, entitled **„Factors influencing the fingerprinting of the polyphenolic profile during the process of elaboration and maturation of red wines",** there are evaluated the intrinsic and extrinsic factors that influence the evolution of the polyphenolic compounds during the whole process of vinification. Also, there were highlighted important information that show the implications of the structural transformations which take place along the processes of elaboration and maturation of wines, as well as the factors that influence the evolution of the polyphenolic compounds during the stated stages.

Chapter III, named **„Influence of the transformations of the polyphenolic compunds during the process of maturation of red wines on their polyphenoic fingerprinting",** analyses the implications of the modifications of polyphenolic compounds on their incidence in the maturated wines. Under these conditions, the polyphenolic composition of the red wines at different stages of storage influence their polyphenolic fingerprinting.

The second part of the thesis comprises Chapter IV, which refers to the own experimental research; in this chapter there are revealed the samples used in the achievement of the research: skins, pulp, seeds and juice, as well as the resulting wines, at different stages of technological evolution; the utilized red varieties were Cabernet Sauvignon, Merlot and Fetească Neagră from the viticultural areas Murfatlar and Valea Călugărească from two years of harvest, 2014 and 2015.

Furthermore, there were presented the used research methods: determination of the total polyphenol content, determination of the antioxidant activity through the DPPH (A_{AR}) method and the chemiluminescence (SA_{HFR}) method, high performance liquid chromatography coupled with mass spectrometry (LC-MS), gas chromatography coupled with mass spectrometry (GC-MS), determination of the volatile profile by use of the Electronic Nose, but also the oxidation of low density lipoproteins (LDL) catalyzed by copper ions.

Regarding the statistical elements that led to the analysis of the results, they were: the analysis of variance (ANOVA) mono- and bi-factorial, the principal components analysis (PCA), the canonical discriminant analysis (CDA) and statistical modeling, by use of multiple regression and the least square method.

The third part of the thesis presents the obtained results and their statistical interpretation. This section is composed of 10 chapters which analyze the values of the evaluated parameters as follows:

Chapter V highlights the comparative results from the point of view of the total polyphenol content (TPC) and the antioxidant activity (A_{AR} and SA_{HFR}) for the grapes of the analyzed red varieties. Overall, this chapter confirms the diverse distribution of the polyphenols contained by the grapes.

Chapter VI presents the value distributions of the analyzed populations and the statistical correlations between CTP, A_{AR} and SA_{HFR} in the case of grape elements. Strong positive correlations were observed between the variables CTP and A_{AR}, CTP and SA_{HFR}, but also A_{AR} and SA_{HFR}, always taking into consideration the Spearman correlation coefficients.

Chapter VII, in a similar way to Chapter V, evaluates the wine samples from the studied red varieties from the point of view of the TPC, A_{AR} and SA_{HFR}; the next step, presented in **Chapter VIII**, consisted in the determination of the statistical correlation of the mentioned parameters. As in the previous case, the wines also showed good correlations regarding the characteristic polyphenol and antioxidant potential.

Further, in **Chapter IX** the variance analysis ANOVA mono- (OneWay) and bi-factorial (TwoWay) was applied. The structure of this chapter was based on the use of ANOVA OneWay corroborated with Tukey test in order to highlight the significant differences for the evolutions of CTP, A_{AR} and SA_{HFR}; the ANOVA TwoWay followed, which was applied in order to highlight the factor and the way their interactions influence the polyphenolic and antioxidant potential of the studied wines. Both the variety-viticultural area interaction, as well as the variety-harvest year interaction demonstrated important implications on the total polyphenol content and on the antioxidant capacity of the analyzed samples.

Chapter X presented the results obtained by high performance liquid chromatography coupled with mass spectrometry; the LC-MS analysis allowed the obtaining of the polyphenolic fingerprints for both the grapes and the resulting red wines. The identified polyphenolic compounds were grouped according to the belonging class: elagic acid, gallic acid and caffeic acid – phenolic acids class; quercetin 3-β-D-glucoside, miricetin, luteolin, quercetin and (-)-catechin – flavonol class; epicatechin and rutin – flavan-3-ol class; delphinidin, peonidin 3-O-glucoside, pelargonidin, malvidin and cyanidin – anthocyanin class.

Chapter XI approaches the statistical modeling method which is based on the multiple regression and on the least square method; the linear models were applied to the data sets obtained in the previous chapter, thus highlighting the various implications and influences of the factors taken into consideration (variety, harvest year, area of origin) on the incidence of the polyphenolic compounds in the grapes and the resulting red wines. In turn, the grapes

constitutive elements revealed different effects that were induced to each of the classes of polyphenols.

In this context, Chapter XII highlighted the way in which the polyphenolic compounds play an important role in the differentiation among samples (variety, harvest year, area of origin) by applying statistical methods different to the one in the previous chapter; these were the principal components analysis (PCA) and the canonical discriminant analysis (CDA). The most important results are obtained from the simultaneous grouping of the samples by variety, harvest year and area of origin, giving the thesis the envisaged novelty character. Also, in this chapter there are presented the preliminary results regarding the identification of some wine authentication markers that are different from the ones identified in the literature.

Chapter XIII evaluates the results determined by GC-MS Headspace of the volatile compounds; moreover, the sensorial analysis done by use of the Electronic Nose confirms the differentiation achieved by GC-MS. The two used methods offer complementary information which help in the complex process of polyphenolic fingerprinting of the analyzed red grapes.

Chapter XIV reveals the applicative potential of some polyphenolic compounds in human health. The research followed the evolution of the cholesterol (LDL) oxidation reaction under the influence of polyphenol addition in the reaction system. The results highlighted the induction of beneficial effects of these compounds by inhibiting the LDL oxidation reaction. Furthermore, the obtained data show a concentration dependent behavior for the studied polyphenols, highlighted by their capacity to remove free radicals from the reaction environment.

The processes of elaboration and maturation of red wines are marked by a complex evolution of their polyphenolic compounds. Thus, the statistical analysis approach along with the data obtained from the polyphenolic fingerprinting highlight important aspects regarding the achievement of authenticity and typicity of variety and area of origin for red wines

In the last part of the thesis there are presented the general conclusions of the documentary study and of the experimental research; these are followed by aspects that show the author’s own contributions, the valorization and dissemination of the results, but also the bibliographic references used in the thesis.