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DOCTORAL THESIS ABSTRACT

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The doctoral thesis entitled "Studies on the evolution of volatile compounds and acids in the production of white wines from Fetească regală and Aligoté grape varieties, in the Bucium wine-growing centre, Iasi vineyard" was motivated by obtaining wines from non-aromatic grape varieties, able to face the fierce competition on the wine market, by obtaining wines with specific aroma profiles.

The rationale for studying the two grape varieties was that the Fetească regală variety covers the largest surface percentage in our country and the wines obtained from international Aligoté are characterised all over the world as semi-aromatic.

The thesis is structured in two main parts, according to current scientific rules, by using as bibliographical material a number of 175 references from the most recent and relevant scientific literature.

The first part is structured in three sub-chapters that present information from specific literature, that briefly describe the influence of yeasts on volatile compounds and major organic acids during alcoholic fermentation.

The second part, " Personal Contributions ", is structured in four chapters to which general conclusions drawn from the studies performed in this thesis have been added.

Chapter three presents the scientific aim for performing this study as well as the objectives followed to achieve it. Thus, the objectives centered on:

- monitoring the influence of yeast on the composition of wines and the main physico-chemical parameters, including: volatile acidity, total acidity, pH, relative density, alcohol concentration, non-reductive substances, non-reductive extract, total dry extract
- qualitative determination of flavour compounds in experimental wines from the two studied grape varieties
- determination of the statistical influence of the factors studied (day of fermentation, technological variant and their synergistic action)
- the aromatic profile of wines using "blind" sensorial analysis in order to identify and quantify the main gustative and olfactive characteristics.

Chapter four presents the organizational and institutional framework in which the research on the study of acids in Aligoté and Fetească regală wines was carried out. The determinations of major organic acids and volatile compounds were

carried out in the Oenology Laboratory of the Faculty of Horticulture, Iași University for Life Sciences "Ion Ionescu de la Brad", in 2015.

Chapter five presents the materials and methods used in the research (the vineyard, the two grape varieties analysed, the experimental wine samples obtained and the yeasts used in the study), the technological variants used in the study, the methods of determination of physico-chemical parameters in must and wine, the sensory characteristic evaluation of the wines and the interpretation of the data through statistical calculations.

In order to accomplish the objectives, two grape varieties were studied, one cosmopolitan - Aligoté and one local - Fetească regală. The grapes were harvested manually in 2015, from the vineyard Iasi - Bucium. The processing of the raw material took place in the company S.C. CASA OLTEANU S.R.L., where the primary vinification also took place. After the qualitative and quantitative reception of the grapes by variety, destemming and crushing were applied. The resulting grape juice was conveyed by means of a fulopump to the press, at which time a double enzyme treatment, a light sulphiting and a tannin treatment were carried out, the resulting grape juice was kept in the press for a pre-fermentative maceration. The obtained must was conveyed in stainless steel tanks from Biomashinostroene by means of a heat exchanger in order to carry out a gravitational settling, followed by its homogenization and the obtaining of the experimental variants:

Aligoté VARIANTS	TREATMENTS APPLIED
Variant A1 – control variant	- no yeast applied - general white vinification technology
Variant A2	- selected yeasts added (Levulia® Esperide) - fermented in low capacity glass demi-johns 50 L
Variant A3	- selected yeasts added (Zymaflore® X5) - fermented in low capacity glass demi-johns 50 L
Variant A4	- selected yeasts added (Levulia® Esperide) - fermented in stainless steel tanks 1000 L
Variant A5	- selected yeasts added (Zymaflore® X5) - fermented in stainless steel tanks tank 1000 L

Fetească regală VARIANTS	TREATMENTS APPLIED
Variant F1 – control variant	- no yeast applied - general white vinification technology
Variant F2	- selected yeasts added (Fermol Candy®) - fermented in low capacity glass demi-johns 50 L
Variant F3	- selected yeasts added (Fermactive® Sauvignon Blanc) - fermented in low capacity glass demi-johns 50 L
Variant F4	- selected yeasts added (Fermol Candy®) - fermented in stainless steel tanks tank 1000 L
Variant F5	- selected yeasts added (Fermactive® Sauvignon Blanc) - fermented in stainless steel tanks tank 1000 L

The obtained grape juices were fermented using available selected yeast, as well as yeasts from wild flora. Daily sampling started from the grape juice phase while monitoring alcoholic fermentation. The last day of sampling was the day after protein stability, when the wine was bottled. Thus two samples of must were taken simultaneously, one for subsequent determination of acids and one for determination of volatile compounds. The samples were stored in controlled temperature and humidity conditions for the determination of organic acids and for the determination of volatile compounds.

For the determination of organic acids and volatile compounds, the samples were transported for analysis to the Oenology Laboratory of the Faculty of Horticulture-Iași, where specialised equipment was used.

Chapter six contains the discussions and results obtained from the study. This chapter is structured into four sub-chapters describing: the characterization of the wines according to their main physico-chemical characteristics, the statistical interpretation of the results concerning the content of the majority organic acids determined, the identification of volatile compounds at different stages of the fermentation process and the sensory characteristics of the studied wines.

From the analyzed parameters, the most important are: total acidity, concentration of reducing sugars in musts, volatile acidity, pH, relative density, alcohol concentration, non-reducing substances, non-reductive and total dry extracts, major organic acids, volatile compounds and sensory characteristics analysis of wines obtained.

The tank-fermented samples showed different values of alcohol proportion for both Aligoté and Fetească regală variety. This can be attributed to smaller losses during alcoholic fermentation due to the lower temperatures during fermentation and to the smaller amount of non-reductive substances in these samples.

The Statgraphics® Centurion XVI platform (Stat Points Technologies, Inc. Warrenton, Virginia, USA) was used for validation of results and advanced statistical analysis. The following statistical analyses were performed: arithmetic mean, variance, standard deviation, Fisher test, multivariate statistical analysis (MVA), principal component analysis (PCA), unifactorial ANOVA, and multifactorial ANOVA.

Multifactor ANOVA testing was applied on the influence of three factors: grape variety, day of sampling and their synergistic influence on the values determined for the organic acid content identified in the present study in Aligoté wines. The contribution of each factor was measured by eliminating the influences of all other factors. Since the calculated probability values are lower than the probability value tested (0.05), the null hypothesis that the variances of the determined values of each identified acid are equal is rejected. This leads to the

acceptance of the hypothesis that the tested factor, i.e. the studied variety and the day of sampling as well as the synergy between the two factors have statistically significant influence on the content of the identified organic acids for a 95% confidence interval. The same results were obtained for Fetească regală. This leads to the conclusion that the values are not constant for wines obtained from the same grape variety, as they are closely correlated with the volume of fermentation and the yeasts used in the vinification.

The statistical procedure of multiple comparison of the means for acids values for the variants under analysis was applied in order to establish statistically significant differences between them on samples taken each day during alcoholic fermentation of Aligoté and Fetească regală. For Aligoté variety, between 153 and 226 pairs of means showing statistically significant differences were found, calculated for a tested probability of transgression of $p=0.05$. For Fetească regală variety, a number of 21 to 227 pairs of means showing statistically significant differences were found, calculated for a tested probability of transgression of $p=0.05$. The statistical method for differentiating or identifying possible homogeneous groups of means was the Fisher test (LSD - Least significant difference).

After determination of volatile compounds by gas chromatography, a variable number of volatile compounds were identified depending on the variety, sampling stage and variants studied. In the Aligoté wines, 90 volatile compounds were identified, including 14 acids, 22 alcohols, 40 esters, 4 aldehydes, 6 ketones and 4 terpenes. Of the 90 volatile compounds, 81 were identified in the A1 control variant. This can be attributed to the different strains in the spontaneous flora, which depending on the species, are characterized by different important enzymatic activities - β -glucosidase, lipase, pectinase, protease, glucanase, xylanase, amylase, sulfite reductase, β -lyase (Tufariello et al., 2021).

Regarding Fetească regală wines, 86 volatile compounds were determined, of which: 14 acids, 22 alcohols, 39 esters, 4 aldehydes, 3 ketones and 4 terpenes. Some of the volatile compounds come directly from the grapes and some are formed as a result of biochemical reactions during alcoholic fermentation.

Fewer acids have been identified in the must, and they are found in higher numbers during alcoholic fermentation and then decrease in the wine content.

Six volatile acids out of a total of 14 acids identified in the samples taken during alcoholic fermentation were determined by gas chromatographic analysis in Aligoté must. In contrast to the Aligoté variety, 14 volatile acids were determined in all variants of wine made from the Fetească regală variety during alcoholic fermentation, with eight volatile acids in the must.

The variation of acids can be attributed to the fact that during the alcoholic fermentation process there is a different concentration and production depending

on the yeast strain that was used on the one hand (Chidi et al., 2015), and on the other hand this can be attributed to the mode of ester formation (Tufariello et al., 2021).

Most alcohols in wine results as by-products of yeast fermentation (Jackson, 2020), through enzymatic degradation of amino acids (Musteață and Furtuna, 2012). The formation during alcoholic fermentation of higher alcohols occurs through the metabolism of mono-saccharides by yeast by simultaneous decarboxylation and deamination of amino acids (Țârdea et al., 2010).

Superior alcohols have an indirect role in the development of an aged wine bouquet. Through reaction with organic acids, they contribute to a wider range of esters found in wine. During fermentation, ester production occurs rapidly under the control of yeast enzymes (Jackson, 2020).

The largest group of compounds identified is represented by esters as they are formed during alcoholic, malolactic, acetic fermentation, but also later during storage and aging of wine (Țârdea et al., 2010).

In both varieties Fetească regală and Aligoté, the ester class recorded the highest number of odorous compounds with 40 esters identified in wines obtained from the variety Aligoté and 39 esters in wines obtained from the variety Fetească regală. The highest number was recorded in the control variants: A1 with 36 and F1 with 35 esters. This is due to the fact that non-Saccharomyces yeasts are described as good ester producers (Musteață and Furtuna, 2012).

During the qualitative analysis of volatile compounds by gas chromatography, some of which are mentioned below. In Aligoté, ethyl decanoate and ethyl dodecanoate esters recorded the highest proportion in the A5 variant. The variants fermented with commercial yeast in demi-johns show lower values compared to the tank-fermented variants. The higher values obtained in the variants where fermentation took place in tanks (A4 and A5) can be explained by the fact that the higher temperatures in demi-johns facilitate the evaporation of volatile compounds in greater quantities during alcoholic fermentation (Sablayrolles, 2019). In the Fetească regală variety, octanoic acid and decanoic acid present the highest value in the control variant (F1). The aromas generated by these acids are honey and beeswax, typical aromas of the Fetească regală variety (Țârdea et al., 2010). In the case of the variants fermented with selected yeasts, octanoic acid values are higher in the tank-fermented variants (F4 and F5) than in low capacity glass demi-johns fermented variants (F2 and F3).

The final assessment of wine quality is the sensory profile. This is widely applied in wine research to describe the effect of factors such as grape variety or wine processing technologies and to study the relationship between chemical and sensory characteristics (Carrau et al., 2017).

In the Aligoté wine, the A4 variant is the one that distinguishes itself from a sensory point of view in terms of minerality, notes of green fruit, mown hay, spices, acidity and more pronounced green/vegetable nuances. The A5 variant did not show up, being a balanced variant, which denotes quality. A2 was highlighted by the most intense notes of honey, wild flowers and dried fruit.

The honey note (characteristic of the variety) is strongest in the F1 variety of Fetească regală. The F5 variety also has the lowest intensity of citrus aromas. The F5 variant shines due to its persistence, minerality, ripe fruit notes. The F3 variant has predominant notes of citrus, exotic fruits, wild flowers and spices. The F2 variant excels in terms of texture, notes of green fruits and mown hay.