ABSTRACT

Key words: Sauvignon blanc, Busuioacă de Bohotin, ageing on lees, amino acids, biogenic amines, olfactive descriptors, taste descriptors

The doctoral thesis with the title **"Research on the influence of the use of some oenological products on the ageing on lees of white and rosé wines, obtained in the Iasi-Copou vineyard"** was developed in order to highlight the fact that the wines obtained by the maturation on lees stage with different oenological products, standing out for its better structure and products, for its better structure and aromatic complexity, due to the release through the autolysis process from the yeast cells of numerous macromolecules, which have a major influence on the physico-chemical composition of the wine.

The grape varieties chosen, from which the experimental samples were obtained, were Sauvignon Blanc and Busuioaca de Bohotin because they were considered relevant for this study, considering that Sauvignon Blanc is a variety from which numerous wines are obtained worldwide, and later go through the maturation stage on fine deposit of yeasts. Busuioaca de Bohotin variety is an aromatic variety, which stands out for its characteristic varietal notes, studied for its potential of ageing on lees.

The thesis was structured in two main parts, in accordance with current scientific norms, using as bibliographic material a number of 200 references from specialized literature.

The first part, **"Current stage of knowledge"**, includes the bibliographic research. In this part, information from the specialized literature is presented, which briefly describes the stage of ageing on yeasts in the technological flow of white and rosé wines, the autolysis process of yeast cells, addition by oenological products in that technological stage.

The second part, **"Personal contributions"**, is structured in four distinct chapters, and the conclusions drawn from the studies and determinations made in this scientific work.

Chapter III briefly presents the organisational framework of the Valeriu D. Cotea" Oenology Laboratory within the Faculty of Horticulture, Iasi, where the researchers were carried out. The study material was also described, plus its area of origin in brief.

Chapter IV presents the purpose and objectives of the scientific work. The established objectives are:
- analysis of the main physico-chemical composition parameters of the wines obtained from Sauvignon blanc and Busuioaca de Bohotin grape varieties;
- the identification, determination and quantification of amino acids and biogenic amines in order to characterize some wine and establish the influence of the oenological products used in the maturation stage;
- analysis of the chromatic parameters of the elaborated wines to establish the influence of ageing on lees stage in the technological flow of their production;
- the sensory evaluation of the obtained wines, in order to establish the degree of influence of the oenological products on the sensory characteristics.

The materials and technology applied to obtain the experimental samples were inserted in this chapter. Also, in this chapter were included the analysis methods used to determine the

physico-chemical characteristics of the wine, the method used to determine amino acids and biogenic amines, the methods used for organoleptic analysis and statistical analysis with methods that allowed the interpretation of the data obtained from a statistical point of view.

In order to fulfill the proposed objectives, two varieties of grapes were studied in the scientific work, one cosmopolitan – Sauvignon blanc and one autochthonous, aromatic – Busuioaca de Bohotin. The winemaking process of the experimental samples was carried out in 2020. Immediately after the completion of the alcoholic fermentation process, the new Sauvignon Blanc wine and the new Busuioaca de Bohotin wine, were racked. 13 experimental samples from the Sauvignon blanc variety were obtained, of which 12 samples were treated with different doses of various ageing product and a control sample, with no additions. At the same time, 13 experimental samples from the Busuioaca de Bohotin variety, 12 samples treated with a different dose of various ageing products and one control sample. Thus, 26 experimental samples were taken into study, as to fulfill the objectives undertaken in the doctoral thesis.

The experimental samples were marked with numbers from 1 to 13, followed by SB, in the case of the wine obtained from Sauvignon blanc grape variety, respectively BB, in the case of the wine obtained from Busuioaca de Bohotin grape variety, the list being as follows:

- V1S/V1B (4.02 g product/ 10 L wine);
- V2S/V2B (4.02 g product/ 10 L wine);
- V3S/V3B (4.02 g product/ 10 L wine);
- V4S/V4B (6.01 g product/ 10 L wine);
- V5S/V5B (4.02 g product/ 10 L wine);
- V6S/V6B (2.01 g product/ 10 L wine);
- V7S/V7B (2.01 g product/ 10 L wine);
- V8S/V8B (8.02 g product/ 10 L wine);
- V9S/V9B (3.01 g product/ 10 L wine);
- V10S/V10B (4.02 g product/ 10 L wine);
- V11S/V11B (3.01 g product/ 10 L wine);
- V12S/V12B (4.02 g product/ 10L wine);
- V13S/V13B (no additions).

For the determination of the physico-chemical parameters, the quantification of amino acids and biogenic amines, the experimental samples were analysed in the Oenology Laboratory of the Faculty of Horticulture in Iasi, using the specialized infrastructure provided.

In the **Vth chapter,** the discussions and the obtained results are presented. This chapter is structured in 3 subchapters that describe and analyse the experimental samples according to the main physico-chemical parameters of the wines obtained by using some oenological ageing products, the level of biogenic amines and amino acids identified in the experimental samples after 12 months of ageing on lees, respectively 18 months, and the sensory evolution of the wines under study.

Among the physico-chemical parameters determined to analyse the effect of oenological ageing products are: alcoholic concentration or ethanol content (% vol.), total acidity (g/L $C_4H_6O_6$), real acidity or pH, sugar content (g/L), total dry extract (g/L), non-reducing extract (g/L), volatile acidity (g/L $C_2H_4O_2$), density (g/cm^3), content in malic acid (g/L), content in lactic acid (g/L), and physical determination of color parameters.

In the second subchapter, the concentrations of 7 biogenic amines and 22 amino acids are presented, determined after 12 months and 18 months of ageing on lees. More precisely, analysis was made of the variations of biogenic amines and amino acids on the 26 wine samples and the effects they induce. Concentrations of the following biogenic amines were determined: ethanolamine, phenethylamine, histamine, tyramine, spermidine, putresceine and cadaverine.

Large variations of biogenic amines were recorded for tyramine and putresceine in the case of all samples obtained from the Sauvignon blanc variety. Tyramine values were highest in samples V12SB, V4SB, V2SB and V3SB compared to the control sample, V13SB, after a period of 18 months of contact with lees. A very important aspect is the fact that the experimental samples were treated with oenological ageing products, which present in their composition inactivated yeasts belonging to genus *Saccharomyces* and significant amounts of mannoproteins, polysaccharides, peptides and amino acids with antioxidant proprieties. Putresceine recorded high values in samples V12SB, V3SB and V8SB, ageing on lees with products that have a high content of polysaccharides, mannoproteins, and in the case of the product applied to V8SB, a mixture of PVI/PVPP and chitosan.

Also in the case of the experimental samples obtained from Busuioaca de Bohotin variety, the highest values were recorded in the case of the biogenic amines putresceine and tyramine. Experimental samples V1BB, V11BB and V2BB recorded high values of tyramine after ageing on lees a period of 18 months, the increases being attributed to different decarboxylation and hydrolysis reactions through which macromolecules released from yeast wall pass into the wine. In the case of samples V5BB, V4BB, V9BB, V11BB and V10BB, they were distinguished by a high content of putresceine, due to the stage of ageing on lees with oenological products containing representative amounts of mannoproteins, peptides, polysaccharides, which are subjected to different reactions and thus some of them pass into biogenic amines.

Regarding the amino acids content of the experimental samples obtained through ageing on lees stage for 12 months, respectively 18 months, from the Sauvignon blanc variety, the values of the amino acids L-alanine, L-leucine, L-valine, L-lysine, L-glutamic acid and L-aspartic acid were the highest in all experimental samples. As in the case of experimental samples obtained by Busuioaca de Bohotin variety, the amino acid L-alanine recorded the highest values, followed by L-valine, L-leucine, L-glutamic acid, L-threonine and L-lysine after 12 months of ageing on lees. The concentration of L-alanine and L-proline increased considerably following prolonged contact with ageing product, which have a high content of polysaccharides, mannoproteins, peptides and amino acids with antioxidant proprieties.

Also, in that chapter, correlations were made and explained from an oenological point of view regarding the variations of amino acids, and how they ranged according to their passage through enzymatic decarboxylation reactions into biogenic amines.

In the last subsection of this chapter, the charts of organoleptic analysis of the experimental samples obtained from the Sauvignon blanc variety, respectively Busuioaca de Bohotin, carried out in 2020, were commented. The organoleptic analysis was carried out at the end of the ageing on lees period. It can be seen that the V9SB and V11SB samples recorded intense vegetal olfactive notes, the distinction being a recorded average of 4 and 4,1. The stage of ageing on lees had a positive contribution to the mineral note, especially in the case of

samples V7SB, V8SB, V11SB, where oenological products were used that contain in their composition important amounts of amino acids, vitamins, mannoproteins, and polysaccharides. Floral notes predominate in samples V8SB, V7SB, V10SB and V11SB. Is observed after the addition of yeasts and prolonged contact with them, a large increase in V13SB in terms of olfactory notes of biscuits and bread crust, with the highest average values being in V4SB, V12SB, V7SB and V6SB.

Regarding to the taste analysis of the experimental samples of Sauvignon blanc, a decrease in the acid sensation is observed after the ageing on lees stage, a fact attributed to the polysaccharides released through the enzymatic autolysis process, which allow delicate balance of the astringency and acidity of the wine after only a few months of contact with the oenological products of ageing.

The experimental samples obtained from Busuioaca de Bohotin variety, through olfactory analysis, recorded a high average for red fruits note, specifying that pleasant and intense notes of raspberry, strawberry were detected, as can be seen in the samples V7BB, V10BB, V8BB, V4BB and V9BB. Also, exotic fruits such as mango, papaya were detected by organoleptic analysis with a high average in samples V9BB, V10BB, V11BB and V4BB. In contrast, lower scores for the spice olfactory note are observed in samples ageing on lees compared with the control sample.

Regarding the taste sensations, the experimental samples of Busuioaca de Bohotin have a tendency of increase the sour note, with an average that varies from 3,7 in V1BB sample to 4,9 in the V10BB sample. V8BB, V6BB, V3BB recorded the highest mean values for the sweet taste sensation. The bitter note was more intense in samples V6BB, V7BB and V8BB. This fact appears due to the composition of the ageing products.

In the chapter VIth chapter, the statistical data obtained by evaluating the influence of ageing products on the compositional and sensory characteristics of the obtained wines is presented. The study from a statistical point of view was based on the analysis of the correlation between the composition of the oenological products, the applied dose of each product in each experimental sample and the sensory characteristics perceived through the analysis, thus the one-way results of the ANOVA test were inserted in this paper.

The classification according to which statistical method was followed and applied, took into account the chemical class of main substances, from the composition of each oenological products of ageing used, but also the dose applied to the 12 experimental samples. Under these conditions, the wine samples presented particularities from a sensory point of view, at the level of intensity of olfactive and taste sensations, depending on the composition of the product used. Due to the production technology, it should be noted that the ageing products are rich in polysaccharides, amino acids, peptides and other high molecular weight compounds. The products show the ability to preserve the varietal notes, to enhance them, and to bring added value through the appearance of new notes such as biscuit, autolyse, crunchy, bread crust specific to the autolytic process, aromatic balance and complexity at taste level.

Also, from a statistical point of view, the multifactorial analysis was performed between the composition of some oenological ageing products and the content of amino acids and biogenic amines in the experimental samples. Tests were carried out, namely the Fischer LSD

test, correlation analysis regarding ageing on lees period, applied dose (g/10L) and nitrogen content of applied products.

The discriminant analysis was used to observe the separation of the results obtained according to the three classification criteria, the type of oenological product, the composition of oenological ageing products and the dose applied to each wine sample. Principal component analysis was applied to differentiate the content of the experimental samples in biogenic amines and amino acids depending on the grape variety from which they are obtained.

The cluster analysis allowed the analysis of the evolution of the 22 amino acids and 7 biogenic amines present in the 26 wine samples, using clusters. It was thus possible to describe and discriminate the evolution of analysed biogenic amines and amino acids after 12 months of ageing in contact with lees, respectively 18 months of contact, according to the doses of oenological products administered.