DOCTORAL THESIS ABSTRACT

Key words: Fetească regală, Sauvignon blanc, β-glycosidases, pectinases, amino acids, volatile compounds, phenolic compounds, sensory characteristics

The general quality of wine, its structure and chemical composition are dependent on the raw material characteristics, alcoholic fermentation particularities and the applied winemaking technology (Losada et al., 2011). Awareness of the major role that enzymes play in winemaking contributes to the development of different strategies for optimizing the production process (Boyer, 1970). Numerous studies (Armada et al., 2010; Arnous and Meyer, 2009; Claus and Mojsov, 2018; Kurbanoglu et al., 2020; Mojsov, 2013; Osete-Alcaraz et al., 2019; Ottone et al., 2019; Pardo ș.a., 1999) confirmed the positive impact of using enzymes in food and beverage industries, in improving the quality of final products and optimization of applied production technologies. In this sense, the purpose established for the preparation of this experiment was to monitor the evolution of wine quality under the action of different enzymatic treatments. Commercial enzymes such as pectinase and β-glycosidase were chosen, recommended to be administered in various technological stages. This work refers to the influence of enzymatic preparations administered before alcoholic fermentation, even if most researchers evaluate their use in different stages of the winemaking process. The oenological products used for obtaining the samples followed the conditions and amendments provided in the International Oenological Codex (OIV) and the Codex Alimentarius (FAO). Monitoring the evolution of some compounds (amino acids, phenolic compounds and volatile compounds) is still a topic of interest in the field of research, there are relatively few studies in this regard.

To perform the proposed aim, the doctoral thesis „**STUDIES ON ENZYMES IMPACT ON WHITE WINES TECHNOLOGY FROM IAȘI VINEYARD**" had the following goals: the elaboration of the experimental plan and conducting experimental samples by it; evaluation of physicochemical parameters of the musts; evaluation of enzymes influence on the physicochemical characteristics of wine samples; study on enzymes impact on the chromatic parameters of wines; monitoring the evolution of the main phenolic and volatile compounds during alcoholic fermentation; monitoring the evolution of amino acids during alcoholic fermentation; evaluation of sensory changes of wines; statistical interpretation of the results and comparison of the data with other scientific papers.

The doctoral thesis is structured in 7 main chapters, as follows:

• Chapter 1 includes general aspects of white wine technology and general information on biochemical processes carried out during alcoholic fermentation.

• Chapter 2 addresses fundamental elements regarding the chemical composition of wine.

• Chapter 3 presents general considerations on enzymes.

• Chapter 4 presents information on the current state of research on the use of enzymes in wine production technology.

• Chapter 5 describes the natural, institutional and organizational framework of research.

• Chapter 6 presents the purpose and objectives of the research, the research material, and the applied methodology.

• Chapter 7 includes results, statistical analysis and discussions following the examination of the influence of enzymatic treatments on physicochemical, chromatic parameters, the concentration of phenolic compounds, volatiles, and amino acids, but also on the sensory characteristics of white wines.

Finally, the doctoral thesis presents the conclusions drawn from the experiment.

The experimental tests were performed at the Oenology Laboratory of the Faculty of Horticulture, within the Iasi University of Life Sciences, in 2018. Two semi-aromatic varieties such as Fetească regală and Sauvignon blanc were chosen, extremely widespread in Romanian vineyards and very appreciated by consumers.

The determination of the physico-chemical, chromatic, sensory parameters, as well as the monitoring of the evolution of the amino acids from the resulted samples was performed at the Oenology Laboratory of the Faculty of Horticulture, Iasi University of Life Sciences. The evolution of phenolic compounds and volatile constituents was realized in collaboration with „Iuliu Haţieganu" University of Medicine and Pharmacy from Cluj-Napoca. Investigations were performed according to specialized literature data and following the norms provided by the legislation in force and the International Organization of Vine and Wine regulations (OIV, 2020).

The following parameters were monitored in this paper: the concentration of fermentable sugars in grapes (refractometric method); titratable acidity of resulted musts and wines (titrimetric methods); *p*H of musts and wines (using a *p*H meter); density of the final samples (densimetric method); the alcoholic strength of the wines (simple distillation method); volatile acidity of wines (by titrimetry); free and total sulfur dioxide content of wines (iodometric method); residual sugar level of experimental samples (Luff School method); total and non-reducing dry extract of the final samples; malic and lactic acid concentrations in wine; chromatic parameters of the obtained wines (using UV-VIS spectrophotometry); the content of the main phenolic compounds in different stages of alcoholic fermentation (by liquid chromatography); the concentration of the main volatile compounds in different stages of alcoholic fermentation (by gas chromatography); the amino acid content at different phases of alcoholic fermentation (using high performance liquid chromatography); sensory analysis of the experimental samples.

The administration of enzymatic treatments showed a minor influence on the physico-chemical properties of the final samples, obtaining multiple homogeneous groups, between which there was no statistically significant difference ($p > 0.05$). Comparable results were also obtained by Moroşanu et al. (2016), Samoticha et al. (2016).

Significant differences between the values of the chromatic parameters of the analyzed samples were obtained, depending on the type of administrated enzyme. All Fetească regală wines showed a high level of clarity parameter, with predominant shades of yellow and red, except V1 sample, which was defined by the green and yellow color. The clarity level was significantly higher in V1 comparing to the rest of the samples ($p < 0.05$). Thus, Fetească regală samples showed the highest colorimetric and tonality difference ($p < 0.05$) in the V1 variant comparing to the control sample. For the second category of samples, ΔE values showed significant decreases ($p < 0.05$). Regarding the Sauvignon blanc variety, the largest colorimetric and tonality difference was obtained between V5 and the control sample. It can be observed that the bentonite treatment determined a significant reduction of the main chromatic parameters (clarity, chromaticity, saturation), but also an important increase of tonality. Thus, based on the obtained data, the major impact of bentonite treatment on the clarity and appearance of wine is confirmed. Similar results have been published by Ducasse et al. (2010), El Darra et al. (2016), Gonzáles-Neves et al. (2013), Kelebek et al. (2007), Kelebek et al. (2009), Main and Moriss (2007).

The analyzed samples showed different variations on the levels of the phenolic compounds, depending on the various characteristics and the type of administrated enzymes. Most of the Fetească regală wines were characterized by a high content of protocatechuic and caffeic acid. Best results were obtained in the V1 variant while control samples registered the lowest concentration in phenolic acids. A significant reduction of phenolic compounds concentrations was generated by the bentonite treatment, except for ferulic acid whose concentrations increased.

Sauvignon blanc samples were characterized by a high content of protocatechuic acid, caftaric acid, *trans*- and *cis*-resveratrol. The treated with bentonite variants registered a significant reduction of the analyzed phenolic acids concentrations.

The administration of enzymatic treatments determined significant differences between the concentrations of the main identified phenolic compounds ($p < 0.05$) (except *p*-coumaric acid, ferulic acid and quercetine), rejecting the null hypothesis that all variables would have equal values. The effects of enzymatic treatments on the chemical composition of wines have been widely studied; numerous authors followed the influence of similar oenological products (Bartwosky et al., 2004; Bautista-Ortin et al., 2011; Fernández González et al., 2005; Lengyel, 2014; Masino et al., 2008; Pardo et al., 1999) and reported significant increases in the phenolic composition of the wine.

In terms of amino acid concentration, Fetească regală wines were characterized by a high content of proline, arginine and alanine. Similarly, some authors (Agustini et al., 2014; Castor and Archer, 1955; Herbert et al., 2000; Stines et al., 2000; Valero et al., 2003) obtained high proline concentrations and arginine. The two compounds are not consumed during alcoholic fermentation due to anaerobic conditions and arginine metabolism. Beltran et al. (2004) reported comparable amounts of asparagine (approximately 45 mg/L), lysine (16 mg/L) and proline (approximately 500 mg/L).

Sauvignon blanc wines were distinguished by a high content of proline, alanine, glutamic acid, aspartic acid and serine, respectively. On the other hand, the lowest amounts were recorded for cystine and cysteine.

Burin et al. (2016) demonstrated the reduction of amino acid levels following the application of various conditioning and stabilization treatments, such as the administration of pectolytic enzymes and bentonite. Numerous authors have monitored the level of nitrogen compounds and its variation during the vinification process (Bergdahl et al., 2012; Carrau et al., 2008; Jules et al., 2004; Pinu et al., 2014; Zhang et al., 2003). Some amino acids, such as tyrosine, glycine or ariginine, were not consumed by the administered *Saccharomyces cerevisiae* yeasts during the alcoholic fermentation of Sauvignon blanc samples, which confirms the previous observations made on white wines by Valero et al., (2003) and Pinu et al., (2014). According to the results presented by Cosme et al. (2016), the synthesis of amino acids in grapes usually takes place at the end of their maturation stage, proline and arginine being the main identified nitrogen compounds, followed by alanine, aspartic acid and glutamic acid, in smaller quantities. In conclusion, the data obtained illustrate an important variation of the amino acid profile depending on the grape variety and the applied enzyme treatment.

Following the analyzes performed by gas chromatography, in the experimental samples obtained were identified over 65 volatile compounds, depending on the grape variety. Thus, in Fetească regală samples were identified 25 esters, 12 alcohols, 12 hydrocarbons, 11 acids, and other compounds (carbonyl compounds, terpenes, nitrogen compounds, volatile phenols etc.). On the other hand, the Sauvignon blanc samples were dominated by esters (20), followed by alcohols (15), hydrocarbons (16), acids (7) and other compounds. The identified volatile compounds may come from the raw material, being transferred to the must during the vinification process or may be formed during the alcoholic fermentation, following the biochemical reactions that take place in the wine. The administration of enzymatic treatments determined significant differences between the concentrations of the identified volatile compounds ($p < 0.05$), being rejected the null hypothesis, according to which all variables would present equal values. The highest concentrations for most compounds were obtained in the V1 variant, in both analyzed varieties. Numerous studies have indicated significant enrichment of wines aroma profile with the administration of various enzymatic preparations (Armada et al., 2010; Masino et al., 2008; Rocha et al., 2005; Roland et al., 2012; Rusjan et al., 2009; Tominaga et al., 1998; Visan et al., 2017).

The bentonite clay treatment determined different changes regarding the proportions of the volatile compounds, depending on the substance's class, grape variety and administrated enzyme preparation.

Regarding the level of carbonyl compounds in Sauvignon blanc wines, the administration of bentonite treatment led to an increase of acetoin (3-hydroxy-2-butanone) and benzaldehyde. Other authors have also reported changes in these compounds levels (Vela et al., 2017; Vincenzo et al. 2015). Numerous authors have studied the impact of bentonite treatment on ethyl esters, obtaining different results. Vincenzo et al. (2015) reported a decreasing trend in the proportion of ethyl esters since these compounds are protein-bound. Lambri et al. (2010) reported a decrease in the content of ethyl butyrate and ethyl hexanoate in the Chardonnay variety. Sanborn et al. (2010) obtained a decrease in the level of ethyl decanoate and phenylethyl acetate in the Gewürztraminer variety and no change was reported on the Chardonnay variety. In the case of the experimental samples obtained, this hypothesis was confirmed on ethyl butanoate and ethyl dodecanoate in Sauvignon blanc wines and ethyl hexanoate, ethyl octanoate, ethyl-3-hydroxybutanoate, ethyl decanoate and ethyl 4-hydroxibutanoate in most of Fetească regală variants.

The olfactory analysis provides important information on the quality of food and beverages. Major organoleptic differences can be observed depending on the type of administered treatments but also on the variety particularities. All wines were appreciated as balanced, with excellent acidity, thus gives freshness and a good texture. In the case of the Fetească regală variety, the control sample was characterized by wildflowers rich aromas, green fruits, hay and discreet vegetable notes. The V1 variant stood out for its fruity taste (ripe fruit) and wildflowers notes, with excellent acidity and good texture. Citrus notes were dominant in the V2 sample, with fruity (exotic fruits, ripe fruits) and floral (wildflowers) shades, slightly phenolic taste and high acidity. V3 and V4 variants were distinguished by delicate fruity flavors (ripe fruit, dried fruit), with freshly mown hay notes and good acidity. The aroma of green fruits was well highlighted in the V5 sample, with exotic and citrus fruits shades, a good texture and a high acidity also. In correlation with the identified volatile substances, it can be considered that the organoleptic profile of Fetească regală wines is especially defined by the presence of ethyl octanoate, 3-methyl butyl acetate, hexanoic acid, 2-propyl acetate and ethyl decanoate. In the Sauvignon blanc variety, the vegetal and mineral character were predominant in the V2 variant, followed by V6, while the lowest levels were perceived on the V1 sample. V3 variant was distinguished by the most intense fruity notes (citrus, exotic fruits, green fruits), spicy, slightly phenolic taste, good acidity and texture. The floral (rose flavor) and fruity (dried fruit) character was appreciated in the V5 sample.V6 variant was appreciated for its sweet notes of honey, wildflowers scent and freshly mowed hay. According to gas chromatography data, 3-methyl-1-propanol, 1,6-anhydrous-2,3,4-trimethyl galactose, 3-methyl-1-butanol, diethyl butandioate, 1-phenyl ethanol and acetic acid have an important contribution in defining the sensory profile of Sauvignon blanc wines.

The bentonite treatment generated a significant reduction in flavor intensity and acidity. Bakker et al. (1999) also reported a significant increase in the intensity of sensory descriptors in treated with pectolytic enzymes samples compared to the control one.

V1 variants presented the highest concentrations of identified amino acids, phenolic and volatile compounds. In conclusion, this data contribute to the development of strategies for optimizing the production process, improving the structure and chemical composition of the final product and its sensory characteristics.

The results confirmed the positive impact of using enzymes on the quality of the final samples, obtaining significantly higher concentrations for most phenolic compounds, amino acids, but also enriching the volatile and sensory profile. Also, the data contribute to the development of strategies for optimizing the technological process to improve the quality of the final product.