# ABSTRACT

Key words: Aligoté, Muscat Ottonel, SBSE-GC-MS, PCA, MVA.

The alcoholic fermentation process of the grape-musts constitute a decisive step in the winemaking process, the most wine components being formed along this process. The yeasts are the agents used to carry out the alcoholic fermentation and are the principal contributors to the sensorial and analytical quality of the wine.

During long time, the alcoholic fermentation was carried out by the indigenous yeasts that are naturally present in the skin covering the grape surface, in the cellar equipment and in the fermentation medium. Lately, became widespread the use of starter cultures of yeasts that are selected either from the grape-growing area where the grapes are harvested or from other viticultural areas, being commercialized in various forms. The use of these selected yeasts allows to improve the alcoholic fermentation efficiency, avoiding stuck or sluggish processes, and yields best quality wines.

The wine aroma is the result of the contribution of a few hundreds of volatile compounds that are classified from an enological point of view according to their different origin in varietal and pre-fermentative, fermentative and finally those compounds formed as a consequence of the ageing process. Also it is known that the elaboration of high quality young wines with expressive and distinctive varietal characteristics is a very actual and stable trend of the wine consumers, that the winemakers want to satisfy.

Some compounds, showing low concentration values in wines, may have undesirable effects in the consumer's body. Among these compounds it has to be mentioned the biogenic amines which in high concentrations are toxic, being responsible for certain central nervous system disorders (such as depression and schizophrenia), cardiovascular and digestive system disorders. Although initially biogenic amines were considered „natural" substances in food, it is well known today that their origin can be associated with deterioration processes caused by microorganism because they can be formed through the decarboxylation of amino acids by certain bacteria and yeasts. For these reasons knowledge of the influence of yeasts on wine key quality parameters is necessary.

This study focuses in the alcoholic fermentation, comparing the wines obtained from a spontaneous fermentation with the indigenous microbiota (used as control) and the wines obtained using starter cultures of seven commercial yeasts strains widely used by winemakers in Romania and other countries of Europe, and one yeast strain selected in the Iaşi grape-growing area by the Research Station of Viticulture and Enology of Iași. All the wines obtained by fermenting grape musts of the Aligoté and Muscat Ottonel varieties, cultivated in the Iași area, with the above mentioned yeasts, are analyzed in terms of physical-chemical parameters and also in terms of aroma compounds and biogenic amines contents, to establish the effect of yeast strains in the composition of Aligoté and Muscat Ottonel wines and their quality.

The thesis is structured in two parts, „State of knowledge" and „Own contributions", made up of seven chapters with a total of 219 pages, 35 tables, 42 figures and color pictures, 223 references titles and 26 attachments. The first part of the paper refers to the current state of addressed knowledge issues, including the introduction and three chapters, and in the second part are presented the own research structured in three chapters followed by conclusions and references.

Chapter I, entitled **Present state of research concerning the volatile compounds in wines**, provides up-to-date information on the general aspects regarding the main volatile compounds from wines, their characteristics, as well as the way they are influenced by yeasts.

Chapter II, entitled **Present state of research concerning the biogenic amines from wines,** is composed of three subchapters that refer to the formation of biogenic amines in food, their catabolism and their presence in wine.

Chapter III, entitled **Present state of research concerning the analytical methods used to determine the volatile compounds and biogenic amines in wines,** presents genera and specific data concerning the volatile compounds and biogenic amines analyses, the extraction methods of aroma compounds from wine, as well as the equipment used.

The part with the own research starts with chapter IV in which are presented the **organizational and institutional frame** where the research for the doctoral thesis took place.

Chapter V underlines the main research objectives, the most modern analysis methods protocols, as recognized by the OIV. Also are described the studied grape varieties, the natural

environment of Iasi and Montilla – Moriles vineyards and the way of obtaining the experimental variants.

Chapter VI presents results from analyzes and discussions on them.

The doctoral thesis ends with the general conclusions (chapter VII) that synthetize the main contributions of the dissertation and the implications of the results we obtained as concerns the influence of some yeast strains to the volatile compounds and biogenic amines content of the studied wines.

The main goal of our study is to evaluate the influence of some selected yeasts strains on the composition of Aligoté and Muscat Ottonel wines from Iasi vineyard.

The main objectives of our study were as follows:

- Analyses of volatile compounds of studied grape-musts.
- Developing a method for differentiating the aromatic profiles of grape-musts by chemometrics.
- Establishing the influence of the yeast strains on the main physical-chemical parameters of Aligoté and Muscat Ottonel wines.
- Establishing the influence of the yeast strains on the volatile compounds composition in Aligoté and Muscat Ottonel wines.
- Establishing the influence of the yeast strains on the biogenic amines composition in Aligoté and Muscat Ottonel wines.
- Establishing the influence of the yeast strains on the phenolic compounds composition in Aligoté and Muscat Ottonel wines.
- Analysis of chromatic parameters of the wines, their color computer simulation and calculation of color differences (ΔE).
- Statistical interpretation of the obtained results.

The differentiation of the musts by their aromatic profile was done by using two grape varieties from Iasi vineyard, Romania (Aligoté and Muscat Ottonel) and two grape varieties from Montilla – Moriles vineyard, Spain (Muscat of Alexandria and Pedro Ximénez). The grapes were harvested at optimum technological maturity under sanitary conditions, and were frozen at -18°C until pressing. After thawing in a refrigerator at 4°C, the grapes were pressed with a laboratory stainless steel press (Ferrari, Italy), without crushing the seeds. The grape-must obtained was

homogenized, centrifuged for 5 minutes at 5000 rpm, and immediately subjected to determination of pH, total acidity and sugar. For the analysis of volatile compounds, several fractions of 100 ml were frozen at -18°C in sterile containers.

The free volatile compounds and the ones that were liberated after acidic hydrolysis were analyzed with a gas chromatograph coupled with a mass spectrometer, after being extracted on a magnetic stir bar, followed by desorption in a thermal desorption unit (TDU).

To study the influence of the yeasts strains on the content of volatile compounds and biogenic amines in wine, two white grape varieties were used (Aligoté and Muscat Ottonel) from Iaşi vineyard, harvest 2013. When they reached technological maturity, the grapes were harvested in plastic crates and after quantitative and qualitative reception, the grapes were destemmed, crushed, and pressed immediately with a hydraulic press. The must obtained was homogenized and divided into nine equal parts; eight lots these way obtained were inoculated with pure cultures of selected yeast strains as follow: lot 1 (A1 for Aligoté wines respectively M1 for Muscat Ottonel wines) was inoculated with commercial yeast strain sold under the name of Fermol aromatic®, lot 2 (A2, M2) was inoculated with commercial *Saccharomyces cerevisiae* yeast strain sold under the name of Cross Evolution®, lot 3 (A3, M3) with Zymaflore X16®, A4 and M4 with Fermol Cryoarome®, A5 and M5 with Fermactive Thyol®, A6 and M6 with Fermactive AP®, A7 and M7 with Fermactive Muscat®, A8 and M8 with $A_2B$ yeast strain selected by Research Station for Viticulture and Oenology, Iasi. The ninth lot was left to spontaneous fermentation and used as control. After the alcoholic fermentation ended, the wines were sterile filtered, sulphited, bottled and deposited in cellar. After six months, the wine samples were analyzed in order to evaluate the physical-chemical parameters and the volatile compounds and biogenic amines content. The main physical-chemical and the biogenic amines analyses were performed in the Oenology Laboratory of the Faculty of Horticulture from USAMV Iasi while the volatile compounds and chromatic analyses were performed in the laboratory of the Vitenol Group, University of Cordoba, Spain.

Wines thus obtained were characterized according their alcoholic contents, total and volatile acidities, reducing substances, total dry extract, non-reducing extract, pH values, density, free and total SO2, by means of physical-chemical analysis.

The major volatile compounds from wines were determined by direct injection in a gas chromatograph with FID detector; the minor volatile compounds were determined by SBSE-TD-GC-MS technique; the phenolic compound – by using spectrophotometry methods, color – by using CIE Lab method while the biogenic amines were determined by using liquid chromatography methods.

In order to obtain the wines, we started from homogeneous raw material, the grapes being from the same parcel and the must obtained from pressing them being homogenized and then divided in equal parts in order to inoculate it with selected yeasts strains.

Taking in consideration that the technological process applied was the same for all experimental conditions, the differences obtained among the analyzed compounds are due mostly to the yeast strains tested in winemaking.

For Aligoté wines, the alcohol content varies between studied yeasts with 1.15% v/v, being between 11.11 and 12.26% v/v; all wines can be considered as dry type wine, exception made for the control wines that were obtained with the indigenous microbiota, which also showed the highest volatile acidity content. It appears that the use of selected yeast strains has led to a reduction of the total acidity to 5.48 g tartaric acid/ L (A7) and the increase of the pH value to 3.73 (A6) compared to the control wine which registered an acidity of 6.21 g/L and pH 3.09. Also, under our experimental conditions, the Aligoté wines produced with Cross Evolution (A2), Fermactive AP (A6), Fermactive Muscat (A7), and A2B (A8) yeasts had a non-reducing extract content lower than 17 g/L, that doesn't allow to frame the wine in DOC wine chategory.

Except for the control sample, all the Muscat Ottonel wines obtained were dry wines, with an alcoholic content that vary between 12.89–14.14% v/v, depending on the yeast strain used. The difference of 1.25% v/v alcohol is due especially to the yeast strain, that uses different quantity of sugar in order to produce 1% v/v alcohol. Also, a small part of the difference may be due to the losses that occur during the turbulent fermentation, when a small amounts of alcohol is released by carbon dioxide. The most extractive wines were obtained by using the Fermactive Muscat, Fermactive AP and Zymaflore X16 yeast strains, with non-reducing extract that very between 20 and 22 g/L.

Concerning the volatile compounds, there have been identified 51 compounds in the grape-must of the four varieties studied, out of which 14 were classified as terpenes and

norisoprenoids, seven as aldehydes and ketones, two as alcohols, seven as benzene compounds, eight as organic acids and 13 as esters. From the 51 volatile compounds identified, three were described for the first time in grape-must.

Based on the relative area of all the individual volatile compounds included in the six groups established and using advanced statistical analyses (multiple variables analyses and principal component analyses) an objective differentiation among musts from different grape varieties was performed.

In the case of Muscat Ottonel wines studied, by GC-FID and SBSE-TD-GC-MS analyses 65 aroma compounds were identified out of which 35 were quantified in absolute way and 30 were tentatively quantified, based on their relative area, which is directly proportional to their concentration. It appears that the differences in volatile compounds composition are more quantitative than qualitative.

In order to evaluate the influence of the aroma compounds quantified to the global aroma of wine, we have calculated the odor activity value (OAV) for each compound. Also, each compound was attributed to one or more odorant series, depending on their aroma descriptors, in accordance with the literature. For this study, floral, fruity, green, sweet, chemical and fatty odorant series were used.

For Muscat Ottonel wines, ethyl butanoate, ethyl hexanoate, ethyl heptanoate, ethyl octanoate, ethyl decanoate, linalool, nerol, octanoic acid, diethyl succinate, isoamyl alcohol, E-2-hexenol and 2,3-butanediol (*levo* form) were the most characteristic aroma compounds with OAV above the unit in most cases.

It appears that all the Muscat Ottonel studied wines are characterized by fruity odor, these odorant series registering the highest values (between 37.32 for M1 and 48.51 for M6) and being formed by four terpenes, eight esters and two polyols.

In case of Aligoté wines, there have been identified 64 volatile compounds categorized in the same six chemical groups as the ones identified in Muscat Ottonel wines. The compounds that characterize the most our Aligoté samples, with OAV above unit in most of the experimental variant, were: isoamyl acetate, ethyl butanoate, ethyl hexanoate, ethyl heptanoate, ethyl decanoate, diethyl succinate, linalool, nerol, geraniol, 2,3-butanediol (*levo*), isoamyl alcohol, E-

2-hexenol, and butanoic acid. Also, in control sample, OAV above unit was registered for isobutanol and ethyl lactate.

The odorant series „fruity" registered the highest values in all the experimental variants except the variant A4 were the series „green" was predominant.

The presence of biogenic amines in wines was associated with numerous undesirable physiological effects. The formation of biogenic amines in wines depends on the presence of certain microorganisms and the presence of the amino acid precursors, the period of contact between the grape-must with the skins, the length of the alcoholic fermentation, the concentration of sulfur dioxide, the pH and the contact time wine - yeasts lees. In the microbial groups, the ability to produce biogenic amines is a characteristic of the strain, being reported a large variation in the type and the amount of biogenic amine products of various strains of the same species.

There have been identified eleven biogenic amines (cadaverine, iso-pentyl amine, histamine, tryptamine, phenylethylamine, ethylamine, ethanolamine, spermine, spermidine, putrescine and tyramine), the total content of biogenic amines in wines varying between 9.29 and 14.41 mg/L in Muscat Ottonel wines and between 4.42 and 20.4 mg/L in Aligoté wines, with the mention that in the control sample were recorded significantly lower values than the experimental variants studied. Whatever the variety or yeast strain used, ethanolamine showed the highest value among all amines quantified.

The total polyphenols index (IPT) or OD280 index expresses the content of total phenolic compounds (phenolic acids, tannins and color substances) in the wine, having values between 3 and 15 in white wines and between 20 and 100 in red ones. All variants studied had low values of this index because the wines were obtained from white grapes, that accumulate small amounts of phenolic compounds in comparison to the black grapes and also winemaking was done without keeping the grape-must in contact with the solid parts of the grapes (skins, seeds) to permit the extraction of these compounds to the wine. The small differences observed among the variants, can be explained by the action of yeasts - by metabolizing the phenolic acid or other processes that should be fundamented by further studies.

Determination of chromatic characteristics showed that the most intensely colored wines were obtained by using Fermactive Thyol® yeast strain for Muscat Ottonel wines and Fermol

Cryoaromae® for Aligoté wines. By contrast, the use of Fermactive AP® yeast provided the wines with the lowest color intensity in both varieties studied. There are also significant color and tone differences between the control sample and the yeast used, regardless of the grape variety used. There are no perceptible visual differences in terms of color between the wines obtained by fermenting Muscat Ottonel must with Cross Evolution® and Fermol Cryoaromae® as well as those from Aligoté fermented with Zymaflore X16® and Fermol Aromatic® respectively Fermol Aromatic® and Fermactive AP®. All the other wines can be visually distinguished.

Finally, the organoleptic analysis made by expert judges, using a hedonic scale of 1 to 10, showed a preference for the wines obtained with the Cross Evolution® yeast and Muscat Ottonel grapes. Wines obtained from the Aligoté musts, fermented with $A_2B$ yeast, were also preferred among all the yeasts tested on this grape variety.