ABSTRACT

The doctoral thesis "***The optimization of the technology of production of the white wines in the Iaşi vineyard by using some sorted yeasts from the indigenous flora"*** represents a continuation of the researches on the wine micro-flora of the Copou Iaşi viticultural center.

The present paper is made of 259 pages, being structured in nine chapters, the first two chapters (the general part) comprising the presentation of the technology of white wines production and the present stage of the researches regarding the use of the yeasts selected from the indigenous flora during the alcoholic fermentation process. Chapters III – IX (the experimental part) include the characterization of the natural environment of the area in which the researches took place and the proper results obtained during the doctoral activity.

The large scale production of the alcoholic drinks, the improvement and maintenance of their quality represents a permanent preoccupation. The alcoholic fermentation of the must represents a very important stage in the technology of production of the white wines, being ensured by the yeasts micro-flora. The eventual composition or organoleptic defects appeared because of a fermentation process that took place in improper conditions can be hardly or never recover during the subsequent evolution phases, negatively influencing the quality and stability of the finite wine. The yeasts species and yeasts strains used for the fermentation contribute to the formation or decrease of certain characteristics of wine quality and composition. For this reason, there appeared the need to use the selected yeasts strains whose oenological characteristics are known during the alcoholic fermentation process.

The main objective of the present paper is represented by the isolation and selection of new yeasts strains from the indigenous flora (adaptation for centuries to the pedo-climatic and grape variety conditions of the vineyard) which, if used in the technological flow, are meant to optimize the alcoholic fermentation process, thus obtaining high class wines that totally reflect the personality and potential of the grape variety that are specific to the Iaşi vineyard.

The main criteria according to which the selection of the yeasts strains isolated for the wine-making biotechnological practice is made are: the degree of foam production, the sedimentation capacity and the type of sediment that is obtained, the alcoholic power, the capacity of relay of the alcoholic fermentation process at different temperatures, the resistance degree to different concentration of SO_2.

By knowing the importance of the yeasts with specific fermentation properties, able to contribute to the producing of high quality wines, the isolation and selection activity comes as a necessity. It is well known that the best wines, characterized by typicity, are obtained when the used yeasts are isolated and selected from the wine-making micro-biota of the respective vineyard.

The researches necessary for this paper have been made at the Iaşi Research – Development Station for Viticulture and Wine Making, during 2005 – 2009.

The natural environment of Iaşi vineyard – Copou wine-making center, fulfils the habitat conditions of the vine, both from the point of view of the soil (litho-morpho-pedological) factors and external (bioclimatic) factors. The analysis of the specific eco-climatic conditions of the Copou wine-making center during 1972 – 2008 (in comparison with the multi-annual values) show an increase of the thermal and insulation regime and a decrease of the hydric regime.

In autumn 2005, mature grapes samples have been harvested from the Fetească albă, Sauvignon blanc and Chardonnay vine plantations of the Copou Iaşi wine-making center. These were processed according to the standard working methodology, obtaining the following isolation sources:

- the washing waters of the grape berries;
- the washing waters of the grape stalk;
- non-sulphited musts;
- non-sulphited musts with different concentrations of SO_2 (50 – 200 mg/L).

14 yeasts strains have been isolated from the washing waters of the grape berries and of the grape stalk, 24 yeasts strains from the fresh grapes must without any corrections of composition and 48 yeasts strains from the grapes must treated with different doses of SO_2 (50 – 200 mg/L).

In order to realize the selection of the biologic material, from the point of view of the fermentation characteristics, the isolated yeasts strains have been preliminarily tested in the laboratory. The testing involved the crossing of two stages.

In order to appreciate the fermentation capacity, during the first stage, glass recipients with a volume of 1000 mL were used, monitoring the foaming degree and the time intervals (hours/days) of the pre-fermenting phase, the tumultuous fermentation phase and the post-fermentation phase (quiet fermentation) of the 86 yeasts strains selected from the indigenous flora of the Copou viticultural center.

Not all the 86 yeasts strains selected from the indigenous flora present a real interest

for wine-making, so that the laboratory testing was decisive in choosing those yeasts that correspond to the practical needs from the oenological area. The analysis of the data obtained in the preliminary test allowed the selection of 31 yeasts strains, eliminating 64 % from the total of the isolated yeasts strains. This percentage includes the yeasts strains that frothed abundantly or that started and later finalized the alcoholic fermentation processes.

During the next stage, the 31 selected yeasts strains have been tested again within the alcoholic fermentation process, in glass recipients of 10 liters, following on one hand the reproducibility of the parameters that were monitored in the preliminary test and on the other hand the obtaining of new information regarding the adherence/non-adherence of the yeasts to the walls of the fermentation recipients, the type of the deposit formed in the end of the alcoholic fermentation process, the alcohol production, the clarity degree as well as the physical-chemical and organoleptic characteristics of the obtained wines

After the last verification, 17 yeasts strains were retained with a reduced foaming, settled deposit and reduced from a percentage point of view, with a superior alcoholic strength, with a good carbohydrates/alcohol efficiency, in case of reduced quantities of unfermented sugars and physical-chemical parameters corresponding to quality dry wines.

Crossing all the stages of taxonomic identification according to the classical methodology (J. Lödder, 1971), that the morphological, biochemical and physiological verification, it was noticed that all the 17 yeasts strains - isolated from the "Fetească albă", "Sauvignon blanc" and "Chardonnay" plantations – and considered to have a potential high-performance within the alcoholic fermentation process belong to the *Saccharomyces* type, having the following distribution according to the species: 8 belong to the *Saccharomyces ellipsoideus* species, 6 belong to the *Saccharomyces italicus* species, and 3 yeasts belong to the *Saccharomyces uvarum* species.

In order to introduce the new yeasts strains isolated from the Iaşi vineyard – Copou viticultural center plantations in the wine-making practice, the main oenological characteristics need to be studied according to the standardized conditions:

- the degree of foam production;
- the clarity of the obtained wine;
- the type of the source sediment formed after the ceasing of the fermentation;
- the alcoholic capacity;
- the capacity to lead the alcoholic fermentation to different temperatures;
- the resistance to different concentrations of sulphur dioxide.

As a whole, the data obtained after the application of the tests for the determination of

the oenological characteristics underlined the following aspects:

- from the point of view of the foaming degree, six yeast are averagely foaming, six yeast are minimum foaming (3 – 5 cm3/L foam), and five are non-foaming;
- according to the type of the sediment, five yeasts formed dusty deposits and twelve of them formed compact deposits;
- concerning the clarity character, five yeast finalized the alcoholic fermentation process with thick wines, two yeast produced opalescent wines and the rest of the yeasts led to clear wines;

The alcoholic capacity was evaluated through the determination of the alcoholic concentration and of the non-fermented sugars at the end of three alcoholic fermentation processes made in identical conditions, by varying only the initial concentration from the must, which is 210, 260 and 305 g/L. It was noticed that all the tested yeasts strains are alcoholigenous, being able to ferment the musts that have high sugar concentrations.

In order to underline the capacity of the tested yeasts strains to realize the alcoholic fermentation at different temperatures, the development of three alcoholic fermentation processes was monitored at different temperatures of 13 - 14°C, 22°C and 35°C for each yeast strains. It was noticed that all the yeast strains have the capacity of starting and finalizing the fermentation at low temperatures of 13 - 14°C, and at a temperature of 35°C most of them did not have the capacity to totally ferment the sugars from the sub-layer, except the S3(150), MNC2 and C1(100) yeasts.

Concerning the resistance to different concentrations of sulphur dioxide, the yeasts strains were tested during the alcoholic fermentation process on grapes must treated with doses of sulphur dioxide of 60 mg/L SO_2, 100 mg/L SO_2, 150 mg/L SO_2 and 200 mg/L SO_2. From the 17 yeasts strains, the following yeasts strains showed a certain sensitivity: F1(100), S4(75), S5(75), BC1, and MNC4. These yeasts strains realized lower alcoholic concentrations during the fermentation processes, according to the increase of the SO_2 dose. One yeast, MNF5, did not start the alcoholic fermentation process for the grapes must treated with 200 mg/L SO_2.

After the determination of the oenological characteristics there have been selected nine yeasts strains considered to be of high-performance in the producing of the quality white wines, namely three yeasts strains from the plantation of “Fetească albă” (MNF6, F1(75), F1(200)), three yeasts strains from the plantation of “Sauvignon blanc” (MNS6, S6(75), S3(150)) and three yeasts strains from the plantation of “Chardonnay” (MNC2, C1(100), C4(100)).

In order to be used in the bio-technological practice, the nine selected yeasts strains

were tested at a pilot level. This stage was imposed because the laboratory studies made for the characterization of the yeasts strains in the alcoholic fermentation process cannot reproduce the multiplication conditions of the biological material from the industrial installations.

The testing of the behavior of the yeasts strains was made within the wine-making station of the Iaşi Research – Development Station for Viticulture and Wine Making, which is endowed with fermentation containers having a capacity of 200 liters and whose construction is similar to that of the containers used at an industrial level.

After the ascertainment of the reproducibility of the fermentation parameters and after a complete analysis of the obtained wines from an oenological point of view, only three yeasts strains were nominated, these being considered to have a high-performance because of their stable characters in the making of the fermentation processes and of the obtained quality wines: F1(200) *Saccharomyces ellipsoideus*, S3 (150) *Saccharomyces ellipsoideus* and C4 (100) *Saccharomyces ellipsoideus.* These are individualized because of their non-foaming character, slightly detachable dusty compact sediment, the clarity of the finite product, high alcoholigenous capacity, resistance to sulphur dioxide in a fermentation environment, as well as the possibility to start and finalize the alcoholic fermentation process at low temperatures.

Thanks to the applicative character of the present paper, respectively the optimization of the alcoholic fermentation process by using some yeasts strains selected from the indigenous flora in order to obtain quality wines the researches continued at an industrial level as well.

The fermentation of the musts at an industrial level was made in tanks of 1000 liters, and for the good deployment of the alcoholic fermentation the following basic conditions were assured: the used musts were cleared and sulphited in order to eliminate the spontaneous micro biota; the selected yeasts were introduced into tanks in quantities in order to ensure even from the beginning the optimal density of yeasts cells/mL meant to assure the start of the fermentation process; the alcoholic fermentation was lead at temperatures of 17 - 18°C.

Within the activity of monitoring of the alcoholic fermentation the starting moment and the duration (hours/days) of the pre-fermentation, tumultuous fermentation, quiet fermentation stages were registered, as well as the dynamic evolution of the main parameters: temperature (t^oC), sugars concentration (g/L), alcohol content (% vol.) and total acidity (g/L $C_4H_6O_6$). At the end of the process, the conditioned wines were analyzed from a physical-chemical and organoleptic point of view.

After the testing of the oenological value of the new selected yeasts strains at an industrial level it was noticed that they can be appreciated as a valuable biological material for

the wine-making practice in the technology of the quality dry white wines. The arguments that come to support this statement are sustained by their fermentation and technological properties:

- the new selected yeasts strains start the alcoholic fermentation after 18 – 20 hours from the introduction of the leavens in the must. The stage of the tumultuous fermentation started after 48 ore and continued for 8 – 10 days, which is the optimum time interval for the producing of a moderate metabolization of the sugars.
- the tested yeasts strains are alcoholigenous, being able to completely metabolize the sugars from the fermentation environment;
- according to the foaming degree, the following yeasts strains: *Saccharomyces ellipsoideus* F1(200), *Saccharomyces ellipsoideus* S3 (150) can be filled in the category of the yeasts with a minimum foaming degree in the first 24 hours from the start of the pre-fermentation stage and the *Saccharomyces ellipsoideus* C4(200) yeast filled in the category of the non-foaming yeasts.
- The yeasts deposits are compact, creamy, hardly detachable;

To these arguments, we also add the capacity of the yeasts strains to ferment the sulphited musts with different concentrations of sulphur dioxide, even of 200 mg/L SO_2, with complete metabolization of the sugars. The obtained wines have a fine, discreet, specific flavor; they are fruity and well harmonized with the other components.

All these data confer a complete image over the oenological value of the new selected yeasts strains, which allows their recommendation in the technology of production of the dry white wines in the Iaşi vineyard.