The Study of the factors that decide the vital - metabolic and reproductive growth of acetic bacteria processes present on grapes, in must and wine

(Abstract)

The work is presented in 204 pages and has the following structure:

Introduction

Chapter I. Research conducted nationally and globally on the ecology of acetic bacteria

Chapter II. Aim, research objectives, working methods and materials used in this research

Chapter III. Results

Chapter IV. General conclusions and recommendations

Bibliography contain a number of 185 scientific papers, treaties and monographies published in Romania and the great wine countries of the world

Aim and research objectives

Acetic bacteria or agents are microorganisms belonging to the souring process of wines, belonging to Acetic bacteria type, family Pseudomonadaceae. They are widespread in nature and are common on overripe grapes, particularly on the moldy ones or attacked by insects. They are also found on equipment in cellars and basements and long known for their role in spreading drosophile.

Acetic bacteria are in any type of wine and they wait for favorable conditions for bacteria to multiply. Acetic bacteria are the only bacteria able to oxidize ethanol to acetic acid in acidic conditions, some („overoxidate") under the same conditions, they even further oxidize acetic acid to carbon dioxide and water.

Acetic bacteria are easily identifiable due to the multiplication as a veil on the surface of the wine. This process causes impairment to the quality ,becoming unfit for consumption as such. When Louis Pasteur published his studies on wine the souring process was the most common disease.

Discovering the acetic bacteria and knowing their conditions of life have enabled the prevention of this deterioration, but unfortunately in the process of wine production in Romania, due to serious negligence, these alterations are still present.

The literature reported in Romania that there are not systematic studies about the factors stimulating or inhibiting which decide vital-metabolic activities - of acetic bacteria specific for wines.

As a consequence, we proposed that during 2005 - 2008, to capture some crucial factors that determine metabolic and reproductive processes of the growth of acetic bacteria on the grapes, in must and wine.

The research program has been materialized on grapes, must and wine within the company, The „Crown Domain" Segarcea and the Laboratory of Microbiology of the Faculty of Horticulture of Craiova. Our research objectives were related to:

1. Highlighting the climate during the years in which wine grapes were obtained in order to produce wine,
2. Highlighting the presence of acetic bacteria on grapes and must

3. Highlighting acetic bacteria during the course of alcoholic fermentation

4. Highlighting acetic bacteria during malolactic fermentation

5.The influence of alcohol concentration and pH on the vital-metabolic processes of acetic bacteria;

6. Influence of oxygen on vital- metabolic processes of vital acetic bacteria,

7. Influence of initial cell number of bacteria remaining on the surface of wine vinegar on the speed of development,

8. Influence of doses of sulfur dioxide (SO_2) on vital-metabolic processes of acetic bacteria

9.The effect of temperature on acetic bacteria growth boundary limit, and thermal destruction limit of these bacteria present in wine.

Results obtained

From studies conducted during the period 2005 - 2008, on factors which decide the vital-metabolic processes of reproductive and growth of acetic bacteria present on the grape, in must and wine, there can be drawn some conclusions and recommendations both in scientific and especially in practical field.

1. The amount of bacteria present on grapes and grape vinegar is dependent on the health of the grapes, which in turn is dependent on temperatures and excess moisture from the atmosphere during the riping process. Grape genetic heritage also plays an important role.

   It is shown that white grape varieties are attacked by gray rot in much greater proportions than grapes whose beans have black and thick skin.

2. Favourable conditions that take place in the vital - metabolic processes of reproduction and growth of yeasts and acetic bacteria are different. The

first can provide the energy required to conduct these processes in terms of anaerobiosis and vinegar bacteria only under conditions of aerobiosis.

During the course of alcoholic fermentation process of white grape varieties (Sauvignon) in lag phase acetic bacteria is relatively at the same level as that recorded to obtain must. The exponential phase of alcoholic fermentation, acetic bacteria experiences a decline at the end of the alcoholic fermentation and registers an increase in the number of bacterial cells.

In red wine, when using relatively low temperatures, $18 – 20^0$ C and $28 – 30^0$ C, acetic bacteria entered the same trend, but the number of bacterial cells is more significant than in white winemaking, as a result of specific technology;

3. Presence of acetic bacteria larger phase lag and at the end of alcoholic fermentation is directly linked to the presence of oxygen,

4. The presence of a larger number for acetic bacteria must (for white wine) and pulp (from red wine), derived from grapes attacked by Botrytis gray is explained by the high initial load of grapes with acetic bacteria, sometimes on the expense of yeasts;

5. When harvesting grapes attacked by gray mold it is necessary to impose severe rinsing operation of the must before alcoholic fermentation, using yeast selected to catalyze this process;

6. During the course of malolactic fermentation of red wines, wine environment provides a different metabolic process of bacteria compared to acetic bacteria. Quantities of oxygen are extremely limited for acetic bacteria, but enough for lactic bacteria. Sulfur dioxide doses are not

annoying for lactic bacteria, but they inhibit the metabolic processes of acetic bacteria.

7. Although the amount of oxygen present in wine during malolactic fermentation is small, it was found that it is sufficient for the survival of acetic bacteria

8.At the end of malolactic fermentation when the operations of transfer in order to let it age in small oak barrels (220-250 liter) the proportion of oxygen in the wine increases, as a result, there is an increase in the number of acetic bacteria;

9. Our study demonstrates that for conducting malolactic fermentation, so necessary for high quality red wine; oenological must know and ensure all factors which determine the conduct of the fermentation, but do not neglect the levers that can prevent metabolic activity of acetic bacteria. Neglecting this point in producing red wine can compromise the quality of wine;

10. Alcoholic concentration in wine, read in connection with pH are two factors influencing the presence and activity of bacteria in wine vinegar. In the initial presence of acetic bacteria in the same number, regardless of the alcoholic concentration of wine, pH values from 2.8 to 3.0 are those which hinder the metabolic activity of acetic bacteria, and only at pH values from 3.4 to 3.5 their metabolic activity can be detected.

11. High alcoholic strength seems to be a barrier in deployment of metabolic activity of acetic bacteria, even at pH values from 3.4 to 3.5;

12. Making wine with higher acidity and then fixed with a pH lower with a higher alcohol concentration (less than 12.5% alcohol), may constitute for the category of high quality dry white wines, a hope that in the case of

an initial restricted number of acetic bacteria cells, these wines can be spared of turning into vinegar;

13. In high quality red wines, for which it is preferably a lower fixed acidity than in the white wines to make them more palatable, it is necessary to join them with other levers to remove metabolic activity of acetic bacteria ;

14. Alone, alcoholic and lowered pH (below 3.0) concentration shall not shelter any type of wine, a possible metabolic activity of acetic bacteria,

15. Oxygen can be considered as a major factor for limiting the vital - metabolic activity of vinegar bacteria, they have a strictly aerobic metabolism, oxygen being used as the terminal electron acceptor during respiration process. Oxygen is therefore essential for multiplication of acetic

bacteria .

16. Increasing the number of viable cells, the vinegar bacteria, and that the accumulation of acetic acid in wine are more dependent on the amount of oxygen present in the wine than the wine alcohol concentration;

17. For oenologist is important to note that during wine storage, particularly in oak vessels, oxygen uptake should be extremely limited, so containers must be kept full. For white wines during storage until final conditioning and bottling is advisable to use stainless steel containers. For the red wines left to age in oak containers of low capacity (225-250 liters) should avoid gaps that can be formed and through which oxygen can put the metabolic activity of vinegar bacteria with adverse consequences.

18. Taking an inhibitor (SO_2) of acetic bacteria activity in the limits accepted by law has no effect;

19. Intense metabolic activity of acetic bacteria, where their presence in a favorable environment, is determined primarily by the smallness of the cells, by the high ratio between surface and cell weight. Bacterial cell surface has therefore a very large proportion of absorption, which is why the processes of assimilation and synthesis, growth and reproduction are conducted in a very short time

20.The initial number of bacterial cells remaining on the surface of wine vinegar positively influences their speed of development as well as the accumulation of acetic acid in wine.

The doubled number of bacterial cells remaining on the wine surface, lead to double quantity of acetic acid in wine. For this reason the oenologist shall take all appropriate measures to limit the number of bacteria present on the surface of wine under $10\text{-}10^2$ CFU / ml;

21. After the appearance of veil on the surface of wine the vinegar bacteria number is amplified, and may reach $10^{11} – 10^{12}$ CFU / ml of wine, being created all the conditions for the process of wine vinegar to intensify;

22. With a large number of acetic bacteria present in the wine veil the vinegar process continues.

As in the wine there are found more quantities of acid acetic , the acetic bacteria although in large numbers, decreases their metabolic processes, as a result the average rate of making new acetic acid decreases. Therefore we can conclude that a wine can , because of high proportions of acetic acid formed, become unfit for consumption, but for the same wine even if this process continues, it can not be completed in such conditions. To obtain vinegar there should be provided with other conditions for acetic bacteria to be able to oxidize all the quantity of the alcohol to acetic acid.

23. Inhibition of acetic bacteria with sulfur dioxide is particularly pronounced. Sulphuric acid causes inhibition of acetic fermentation and after a while it leads to destruction of acetic bacteria;

24. Antiseptic effect of SO_2 on acetic bacteria (and other microorganisms on grape and wine) is due to sulfuric acid (H_2SO_3), inextricably linked (active SO_2) and not to ions and the less to sulfuric acid compound,

25. Antiseptic action of SO_2 varies with the age of cell bacteria cultures, temperature, pH and composition of the environment (especially aldehydes and alcohol cultures);

26. Free SO_2 doses below 10 mg / l, without interference of other means (filtering microbiological) shall not protect wines against carrying vital metabolic processes of microorganisms, in our case the acetic bacteria,

27. In the white wines and especially in the red ones providing free SO_2 doses about 15-20 mg / l protects wines sufficiently. Free SO_2 doses above 20 mg / l, especially in red wines offer more effective antimicrobial protection, preventing the normal completion of other stages of wines quality.

28. Temperature is a determining factor; souring of wines progresses more rapidly as the temperature is higher, volatile acidity in the early alteration is two times higher at 28^0 C than at 23^0 C and twice faster at 23^0 C than at 18^0 C;

29. It is very important to understand that in the oenological practices, the optimal growth temperature for acetic bacteria is between $25 – 35^0$ C. At temperatures below acetic bacteria may still be active at 10^0 C. As a result it is difficult to draw a strict temperature range in which these bacteria can

grow, temperatures used during the making of wine seem not to affect the acetic bacteria growth;

30. The growth limit of acetic bacteria is influenced by temperature. Temperatures of 8-12^0 C – maintained while preserving white wines, regardless of their alcohol concentration, may instead maintain the vital metabolic processes of acetic bacteria. Temperatures above 12^{0}C and up to 40 – 45^{0}C stimulate the multiplication process of acetic bacteria and increase the metabolic processes by which wines enrich in acetic acid, and may reach a content that make them unacceptable for consumption.

In red wines, regardless of their alcohol concentration temperatures limiting the growth of acetic bacteria are somewhat higher, 12 – 17^0 C. Over that temperature, especially at low alcohol wines, and up to 40 – 45^{0}C the growth of acetic bacteria is not hindered, wines accumulating large quantities of unacceptable acetic acid ;

31. Temperatures of 30 – 55^{0}C does not contribute to the destruction of acetic bacteria, but rather contribute to their proliferation, both for white wines and the red ones, regardless of their alcoholic strength. It seems however that a wine with higher alcohol content leads to a lowered temperature to destroy acetic bacteria. At an exposure of 5-10 minutes at a temperature of 55 – 60^{0}C there is a decrease in the number of bacterial cells.

In all cases, when white and red wines, regardless of alcoholic strength, are maintained at temperatures of 60 – 65^{0}C for 2 to 10 minutes, the acetic bacteria cells are inactivated;

32. The research studies reveal that acetic bacteria are part of the micro flora present on grapes, in must and wine.

Knowing the factors that determine the vital metabolic processes - gives us a chance to take effective measures to ensure that these processes do not take place, thus ensuring high-quality wines, with the minimum content of acetic acid which can be caused, if it is possible, only because of their alcoholic fermentation of sugars present in must.