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Study of malolactic fermentation. Conditions of adding lactic bacteria

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Abstract: *Malolactic fermentation is caused in red wines when alcoholic fermentation is ended. The great importance of this fermentation is the capability to improve the organoleptic characteristics of wines. In order to cause malolactic fermentation we used lactic bacteria and especially the kind of Bacteries lactiques OEnococcus oeni. Several factors influence the action of bacteria and of course the progress of malolactic fermentation. Especially in the present article we studied the following conditions: Change of temperature, start of malolactic fermentation without addition of lactic bacteria, addition of double quantity of lactic bacteria.*

Key words: *Malolactic fermentation, lactic bacteria, red wines*

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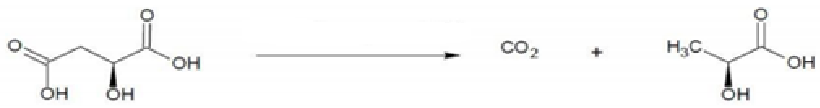
I. Introduction

Malolactic fermentation (MLF) is an integral part of the whole winemaking process. More specifically is described as a secondary fermentation. It does not take place parallel to alcoholic fermentation but after. It is used mainly in red wines (rarely in whites) which have the characteristic of high acidity [1]. Malolactic fermentation is controlled of a special kind of bacteria which are called malolactic bacteria. Chemically during the process of decarboxylation is occurred from L-malic acid ($C_4H_6O_5$) into L-lactic acid ($C_3H_6O_3$) (Figure 1) [2].



L-malic acid $\rightarrow$ CO_2 + L-lactic acid
Figure 1

The importance of malolactic fermentation is that during the whole process we observe (mainly expert tasters) improvement in organoleptics characteristics of wines [3]. High quantity of malic acid is correlated with tart taste [4]. So we can notice reduction of dart taste and increase of aroma complexity (mainly during production of flavor compounds) [5]. Besides that the final product has better microbial stability. However if the process of malolactic fermentation is not efficient controlled the danger of production of undesirable aromas in wines is high. Others compounds of wine e.g. antioxidants do not affect through malolactic fermentation [6].

The bacteria that are correlated with malolactic fermentation belong mainly to the Pediococcus, Lactobacillus and Oenococcus strains [7]. Researchers have shown that Oenococcus Oeni are the most capable for the whole process [8].

A wide range of factors affect malolactic fermentation. Grape cultivar, the bacterial culture, and the bacteria adding conditions are the most important. In this article the parameter of temperature and quantity of bacteria was examined in two different types of wine (Arachovitico and Cabernet Sauvignon).

II. Wine Samples

Malolactic fermentation was monitored in two different Greek wines, Cabernet Sauvignon and Arachocitico. The characteristics are the following:

Cabernet Sauvignon: International red variety originating from Bordeaux, France, the cultivation of which was considered necessary to improve the red wines produced by some Greek varieties. In Greece, it was first cultivated in Metsovo and its cultivation quickly spread to several areas throughout Greece (from Crete to Thrace), allowed or recommended in 23 prefectures.

Arachovitico: Local variety, which is cultivated in the Boeotian town of Arachova, located below Parnassos. There are 60-year-old vines in the area, but for the past 20 years, in an effort to combat phylloxera, old vines have been phased out and replaced with vines grafted onto hardy American roots [9].

III. Method - Experiments

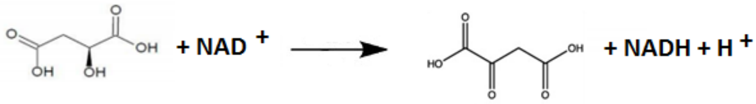
The main chemical ways in order to monitor malolactic fermentation are enzymatic spectrophotometric methods. Malolactic bacteria of genus OEnococcus oeni were used to initiate malolactic fermentation. There were produced in France by St-Simon-France and purchased from a Greek chemical company. The amount of bacteria purchased was 25 g, a quantity capable of causing malolactic fermentation in 2500 L of wine [10].

In order to calculate the percent of malic acid that turns to lactic we used kit for the determination of malic acid, which was procured from Boehringer Mannheim. The reagents were used in the form they have, except NAD, which was dissolved in 6 ml of deionized water. More specifically the kit contained the following:

- glycyglycine buffer pH approx 10,0 L- glytamic acid approx 440 mg
- 210 mg NAD lyophilizate
- glutamate-pyruvate transaminase suspension
- L-Malate dehydrogenase solution
- L-Malic acid assay control

The measurement of malic acid concentration is performed with the Boehringer Mannheim assay kit. This is a spectrophotometric method (official method).

To be more specific the method is based on the fact that in the presence of the nicotinamide-adenine dinucleotide (NAD), L-malic acid is oxidized to oxalic acid, in a reaction, which is catalyzed by L-malic dehydrogenase (L-MDH). The equilibrium of the reaction is shifted towards the malic acid side. Removal of the oxaloacetic acid from the reaction medium shifts the equilibrium of the reaction (Figure 2) in the direction of oxaloacetic acid formation (to the right). In the presence of L-glutamate oxaloacetic acid is converted to L-asparagine reaction which is catalyzed by glutamate oxaloacetate-transaminase (GOT) [11].



L-malic acid + NAD^+ → Oxaloacetate acid + NADH + H^+

Figure 2

The amount of NADH formed corresponds stoichiometrically to the amount of malic acid, according to reaction (1). The increase in the amount of NADH is determined by measuring the absorbance at a wavelength of 340 nm. The experimental procedure is described in the instructions in the Boehringer Mannheim kit, with the only difference that the sample is diluted 1/10, because our red wine samples have very intense color.

After 3 minutes, measure the absorbance A1 of the blank and the sample solution. Then add to both solutions 0.010 ml LMDH and after 5 minutes measure the absorbance A2 of the blank and the sample. Then we calculate:

$\Delta A = (A2 - A1)$ sample - $(A2 - A1)$ blind

The concentration of malic acid C is calculated based on the formula:

$C = (V \times M_r \times \Delta A) / (\varepsilon \times d \times v \times 1000)$ (1)

Final formula for calculating the concentration of malic acid in g / l

Where:

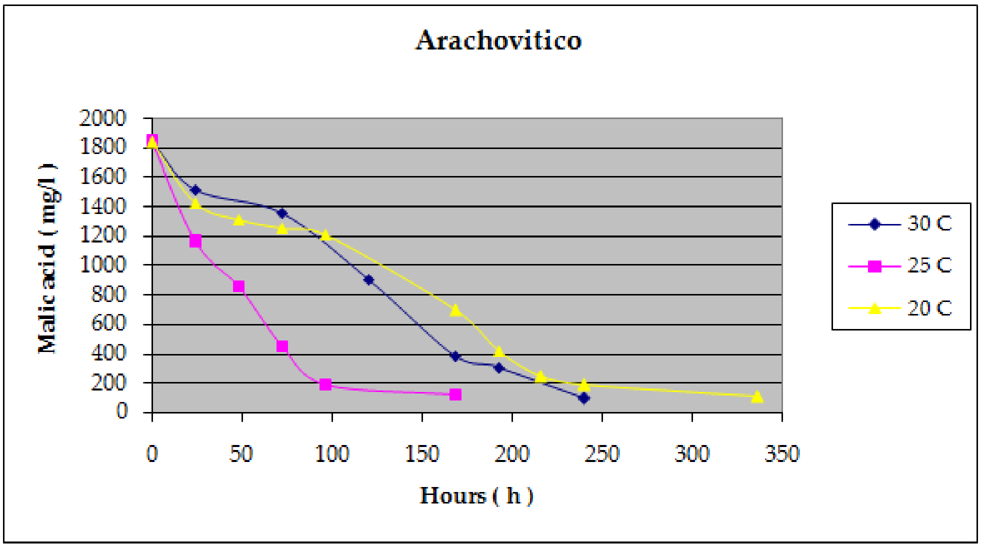
- V / Final volume of sample in ml (here 2.22 ml)
- v / Volume of wine sample in ml (here 0.010 ml)
- Mr / Relative molecular mass of malic acid (134.09)
- d / Optical cell path in cm (1 cm)
- ε / Absorption coefficient of NADH

Micro-fermentations were performed. Specifically, 250 ml of wine from each sample was used in each cycle of experiments, which was inoculated with the corresponding amount of lactic acid bacteria suggested by the literature. The fermentations evolved into 500 ml conical plastic bottles, which were capped with cotton and aluminum foil, but were not sealed. The samples of red wines used (Cabernet - Arachovitico), were taken from wine tanks as soon as the alcoholic fermentation was over. The specific samples were not sulfated and stored in the refrigerator and specifically in storage at 4^o C. The effect of each factor (temperature, quantity of bacteria) in the course of malolactic fermentation, was examined as a function of time. As starting point of the fermentation was considered to be the moment when plastic bottles were placed in the water bath began after their inoculation. The ending point of completion of fermentation is considered, according to the literature, the moment when the concentration of malic acid approaches the value of 100 mg/l.

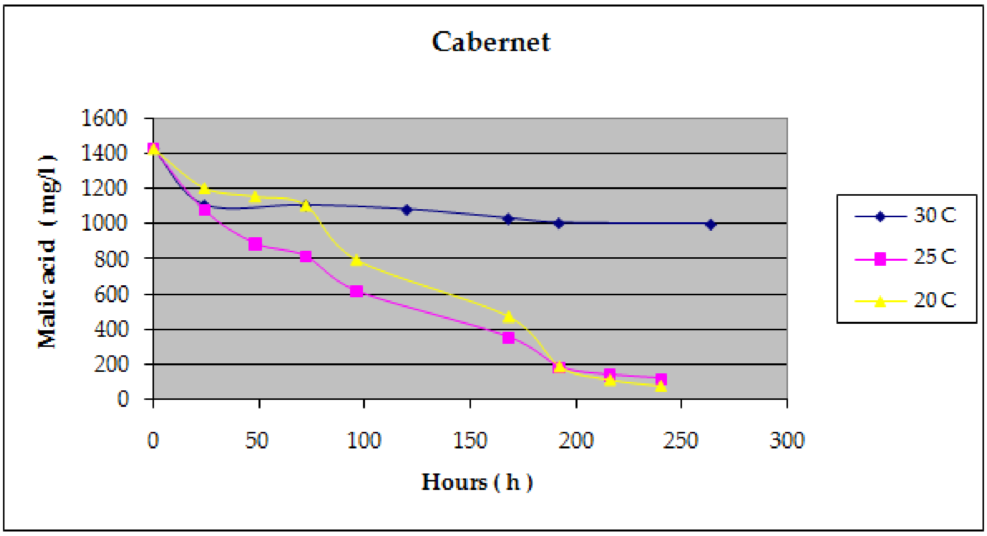
IV. Primary - Results

Effect of Temperature

Malolactic fermentation was examined at three different temperatures 20° C, 25° C and 30° C. At regular intervals, 5 ml samples were taken, in which the concentration of malic acid was measured. The corresponding graphs (graph 1,2) are as follows:



Graph 1

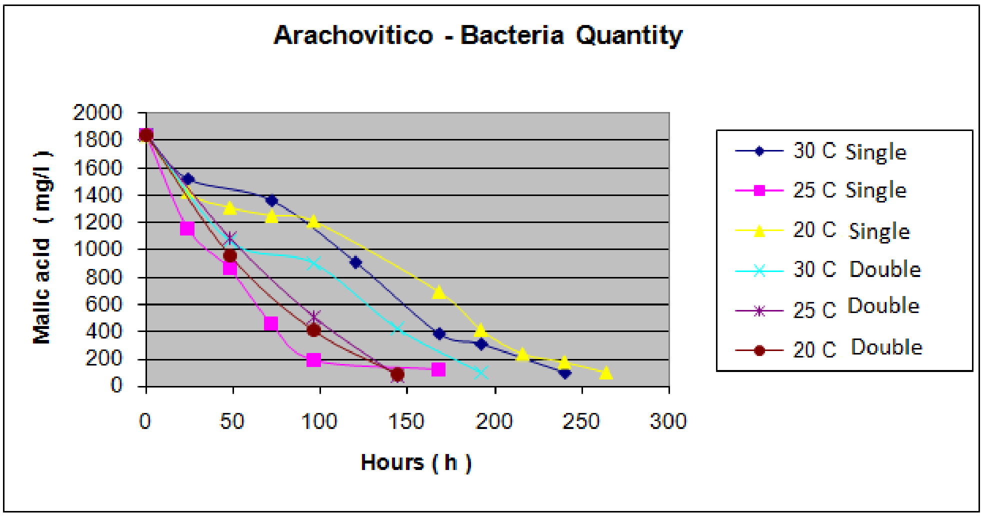


Graph 2

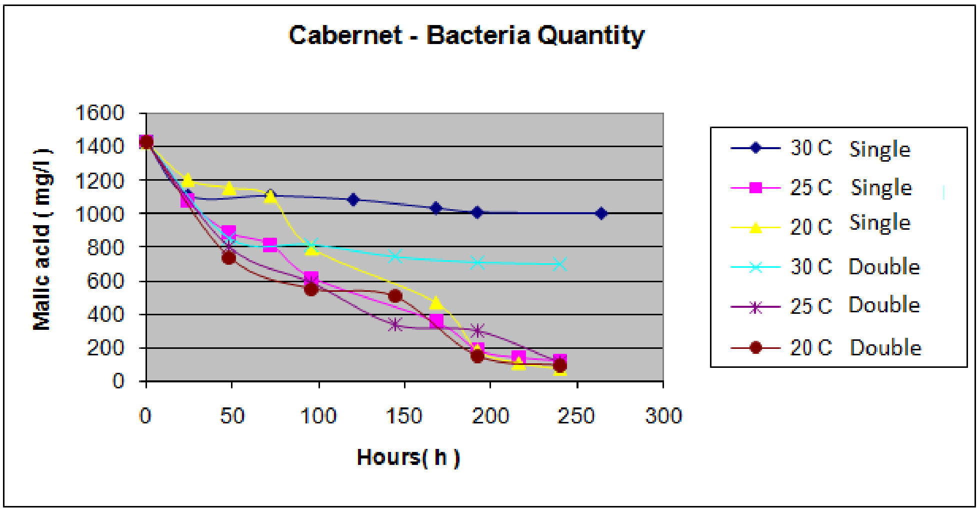
Temperature is one of the main factors influencing malolactic fermentation. The temperature zone in which fermentation can take place is from 18^o C to 35^o C, in an environment of course that contains the appropriate nutrients. Studying the comparative diagrams we observe that the ideal temperature for our samples is that of 25^o C, at which the fermentation is completed faster. Impressive is the fact that for the Cabernet sample and for the temperature of 30^o C the malolactic fermentation is sent to the value of 1000 mg/l, something that may be related to the special characteristics of this variety or to the fact that the 30^o C approach the upper limit of the ´´ temperature ´´ zone.

Quantity of bacteria

In this case first of all the process of malolactic fermentation was investigated without the presence of lactic bacteria. In this case the concentration of malic acid was constantly constant (arachovitico 1800mg/l, cabernet 1600 mg/l) and the fermentation did not initiate. In the second step wine samples were inoculated with the double amount of lactic acid bacteria compared to the amount we used, which was according to the instructions on the packaging of the lactic acid bacteria. Then the experiment was repeated by thermostating the samples at temperatures of 20° C, 25° C and 30° C. Then the new concentrations of malic acid were measured. The corresponding graphs (graph 3, 4) are as follows:



Graph 3



Graph 4

Studying the comparative diagrams for the Arachovitico sample at all three temperatures, we find that by adding the double amount of lactic acid bacteria, malolactic fermentation is completed earlier, compared to the corresponding experiments involving the addition of a normal amount of bacteria at temperatures of 20° and 30° C and about at the same time at the temperature of 25° C. In fact, for the temperature of 20° C the fermentation is completed in about half time. The existence of higher amount of bacteria does not work competitively. There is sufficient food for them to grow and then initiate malolactic fermentation.

Examining the comparative diagrams for the Cabernet sample at all three temperatures, we find that by adding twice the double amount of lactic acid bacteria, malolactic fermentation is completed at the same time as the corresponding experiments involving the addition of a normal amount of bacteria at 20° C and 25° C. For the temperature of 30° C the fermentation is also inhibited but at the concentration of 700 mg/l.

V. Results – Discussion

As mentioned in the theoretical part, malolactic fermentation is perhaps the only way to improve the organoleptic characteristics of red wines and do not affect other parameters e.g. antioxidant activity [12]. It takes place after the end of the alcoholic fermentation. It depends on a number of factors, both endogenous (related to the specific wine) and exogenous (related to the conditions of the environment).

Consequently we can mention the following: The optimum temperature for the contraction of malolactic fermentation is 25^o C. At the temperature of 30^o C, depending on the sample that we have, we may observe total suspension of malolactic fermentation. Without the addition of lactic bacteria we do not observe initiation of malolactic fermentation. If we want to suspend or retard malolactic fermentation we can fridge wine samples. Any lactic bacteria that could exist in the wine are inactive due to the low temperature of 4^o C. On the contrary the addition of double quantity of lactic bacteria leads gradually to faster completion of malolactic fermentation.

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