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Assessment of the Development of Malolactic Fermentation of Wines Produced in Albania

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Abstract

Malolactic fermentation occurs as a result of the action of lactic bacteria that affect the reduction of the acidity of the wine, it is responsible for the formation of the taste and aroma components of the wine. This process must be controlled since its spontaneous initiation and development brings the risk of the formation of compounds that lead to the deterioration of the physico-chemical and organosensory indicators of the wine. Malic acid is biodegradable, this causes biological stabilization that must occur before the wine is bottled. For malolactic fermentation to occur, the wine must have less than 14% vol alcohol, pH ≥ 3.3 and low clarity. The purpose of the study is to document the development of malolactic fermentation in Albanian wines and to increase the awareness of producers about the importance of the process in the quality and stability of the wine. 50 wine samples were taken for analysis from these 32 red and 18 white wines, all locally produced and bottled, each of them was subjected to physical-chemical, organosensory evaluation according to Regulation (EC) No. 479/2008; Analytical methods OIV and assessment of malolactic fermentation progress with paper chromatography. The assessment showed that only 40% have completed the FML, 36% are in the process, 24% have not yet started the FML. In some of them, fatty aromas and fermented vegetables were detected, which indicates the spontaneous development of the process, in 14% of them, the presence of diacetyl and high fluoric acidity was detected, which indicates that FML was not performed in the right time and manner. In order to have the quality and durability of the wine, it is important that this process is carried out under control before the wine is bottled.

Keywords: Malolactic fermentation; wine; paper chromatography.

1. Introduction

Malolactic fermentation (MLF), the enzymatic decarboxylation of L-malic acid to L-lactic acid and carbon dioxide, is the important secondary fermentation conducted by wine bacteria [1]. MLF is the bacterial-driven decarboxylation of diprotic (S)-(-) -malic acid (sharp green apple character) to monoprotic (S)-(+)- and/or (R)-(-)-lactic acid (softer, yoghurt character) and carbon dioxide. It usually occurs after alcoholic fermentation, but studies investigating advantages of cofermentation (inoculating yeast and lactic acid bacteria simultaneously) are ongoing [2]. The main parameters that influence bacterial activity are pH, temperature, ethanol and SO_2 concentration [3]. It has been proven that lactic bacteria work better in must than in must-wine (after alcoholic fermentation), especially when the conditions for cell growth are not favorable [4] ,[2], [5], [6]. In winemaking, MLF is one of the most difficult processes to control and can affect the final aroma and taste balance of the product by modifying fruit derived aromas and producing aroma active compounds [7]. It has been observed that wines that have undergone simultaneous alcoholic fermentation /maloloctyic fermentation tend to be less buttery and are fruitier [8], [9], [10].

One of the main effects of the malolactic fermentation is deacidification, which is particularly desirable for high acid wine produced in cool climate regions.[11]

MLF is often spontaneous and difficult to stop in red winemaking, possibly due to the higher temperatures involved and/or through the activities of indigenous lactic bacteria found in cooperage. Producers will allow it to proceed or actively encourage it early in the life of

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a wine in order to prevent it happening in the bottle. After undergoing a malolactic fermentation, and as long as residual sugar levels are low, the wine is relatively microbiologically stable.

Lactic acid bacteria are important in winemaking for two major reasons, they affect wine quality and they affect the economics of wine production [12]. Heterofermentative bacteria also metabolize citric acid to acetic acid, lactic acid and carbon dioxide [13]. Lactobacillus and Pediococcus are capable of malolactic fermentation, but can produce undesirable characteristics – especially if the fermentation happens in the bottle. As with refermentations after bottling caused by the presence of yeasts, malolactic fermentations in the bottle can cause the production of carbon dioxide with off odors of vegetative, vinegar, mousy or geranium characteristics if sorbic acid is present. O. oeni is heterofermentative and utilizes glucose via the phosphoketolase pathway, resulting in approximately one-sixth carbon dioxide, one-third ethanol, acetic acid or acetaldehyde and the remainder as lactic acid [12]. However, if malic acid is present, this will be degraded before any glucose molecule. The survival and growth of the bacteria are dependant on certain conditions, such: pH of 3.3–3.5, no free SO_2, total SO_2 less than 50 mg/l and a temperature of 18–25 ºC. The SO_2 concentration did not constitute a limiting factor for bacteria, the dose of 50 mg/L SO_2 is considered standard for red vinification and is compatible with co-inoculation, provided that bacteria are inoculated at least 6-8 h after SO_2 addition in order to allow the SO_2 to combine completely [14]. Once one tank has fermented, the lees may be used to inoculate other tanks if the wine showed no high increase in volatile acidity or other organoleptic defect. The bacteria also prefer an environment free of sorbic acid with low levels of glucose present [15]. MLF induced concurrently with alcoholic fermentation can reduce the vinification time. The use of co-inoculation to terminate MLF faster with respect to traditional winemaking can help protect the wine from spoilage. Early inoculation of malolactic bacteria in wine may be a way to reduce the risk of volatile phenol production by Brettanomyces [16], this leads to an unwanted increase in volatile acidity and a negative impact on alcoholic fermentation due to the simultaneous action of yeasts and lactic bacteria. We find this problem during the early inoculation of bacteria during wine production, this technique is used very little by wineries, as there is a risk of excessive production of acetic acid from sugar metabolism under the action of lactic acid bacteria..

2. Material and Methods

The purpose of the study is to identify the conditions of wine production and the correct follow-up of the technological scheme. FML is considered one of the most important processes for creating the taste of wine. Often times the producers bypass this process and in most cases the wine spontaneously undergoes the FML process. In order to assess the incidence of this process in the wines produced and marketed in Albania, we randomly took about 50 wines produced with autochthonous varieties from local producers, from these 32 red and 18 white wines, all produced and locally packaged. these wines were each subjected to physical-chemical, organosensory evaluation according to Regulation (EC) No. 479/2008; Analytical methods OIV and evaluation of malolactic fermentation progress by paper chromatography with Whatman No. 1 chromatographic paper according to the method of Ribereau-Gayon and Peynaund. The methodology aims to extract organic acids among them to identify the presence or absence of malic acid in the analyzed samples. The extraction is done with a solution: n-butanol + ½ acetic acid in the presence of bromphenol blue indicator.

3. Results and Discussion

Uncontrolled FML is accompanied by an inevitable increase in fluoric acidity mainly when pentoses are fermented. Other components whose presence is greatly influenced are diacetyl and acetone, which increase two to three times.

According to some authors, the production of acetone and diacetyl is related to the use of citric acid by lactic bacteria. This directly affects the quality of the wine as the increase in diacetyl adds the aroma and taste of butter to the wine. MLF itself is a decarboxylation and as such makes possible the production of a mild acid such as Lactic Acid from Malic Acid, thus significantly reducing the total acidity of the wine. In order for MLF to occur under inolulation conditions, the alcoholic fermentation must first be completed and we must be in certain conditions of pH $\geq$ 3.3; temperature (20-25°Celsius); sugar content 0-2g/l. If MLF occurs spontaneously, the quality of the wine will be questionable depending on the specific conditions in which the wine is located. The influencing factors of the process are:

- Technological: (production or alcoholic fermentation in the presence of wine or not, also the degree of acidity of the wine),
- Physical: (the optimal temperature for the action of Leuconostoc is 25-30°C, while Lactobacillus is 25-35°C. In both cases above 35°C and below 9-10°C Fermentation does not occur)
- Chemical: Intensive aeration favors the beginning of the process since lactic bacteria are microaerophilic, but saturation with oxygen delays but does not prevent the process. CO_2 helps the bacterial breakdown of Malic Acid very quickly.
- Sulphitisation of wine plays an inhibitory role on FML agents, lactic bacteria are much more sensitive than yeast in relation to the presence of SO2. If SO_2 is added to dry and yeast-free wine, a small amount of it is enough to block the biological deacidification of the wine.

From the physicochemical evaluation of importance for the quality of the wine, we distinguish the total acidity, the alcoholic content and the SO_2 content. The paper chromatography rule is based on the evidence of the presence or not of Malic Acid, this is also related to the organosensory changes of the wine.

Table 1. Analytical control results for Alcoholic degree, total acidity, SO_2 content and MLF determination.

White Wine Code	Region	Alcoholic degree \in % vol a.a	Total acidity (gr/lt ac.tartric)	Free SO2	MLF Process
VIOB	Tirane	11.5	5.7±0.2	95±0.7	No
RIEB1	Leskovik	11.5	6 ± 0.2	146±1.4	No
SHB1	Tirane	11	7±0.3	141±0.7	No
Tokaj	Gjirokaster	12.5	6±0.5	159±0.7	Process
SHB2	Gjirokaster	11.5	6±0.2	199±0.7	No
RIEB2	Leskovik	11.3	5±0.4	61±0.7	No
RIEB3	Durres	11.1	6±0.1	41±0.7	Yes
RIEB4	Leskovik	11.4	5±0.07	53±0.7	Yes
SHB3	Tirane	11.1	6±0.6	147±0.7	No
TOK1	Librazhd	11.1	6±0	163±0.7	No
TOK2	Librazhd	12.5	5±0.02	135±0.7	No
TOK3	Librazhd	12.5	5.4±0.2	101±0.	No
TOK4	Librazhd	11.5	6±0.2	167±0.7	No
TOK5	Librazhd	11.5	6±0.05	79±0	No
TOK6	Librazhd	11	5±0.02	123±0	No
TOK7	Librazhd	12.5	7±0.19	191±0	Process
TOK8	Librazhd	11.5	7±0.3	145±0.7	Process
MOS1	Gjirokaster	11.5	5±0.4	339±0.7	Process
Red Wine Code					
KRTSH 1	Berat	13.2	5.6± 0.1	115±0.7	Yes
MRLT1	Berat	13	5.6±0.1	239±0.7	Yes
MRLT2	Lushnje	12.7	8±0.02	101±0.7	Yes
PNR 1	Leskovik	12.5	7.1±0.1	539±0.7	Process
MVRD 1	Leskovik	12	6.2±0.3	295±0.7	Process
SHSHZ 1	Skrapar	12.5	6.7±0.3	221±0.7	Yes
MVRD 2	Leskovik	12.5	5.3±0.02	139±0.7	Yes
KBRNT 1	Durres	13.1	8.7±0.03	159±0.7	Yes
VKKZH1	Librazhd	12.5	6.4± 0.03	587±0.7	Process
VKKZH 3	Librazhd	12.5	8±0.2	251±0.7	Process
MRLT 3	Tirane	13	5.78±0.1	113±0.7	Process
SRZZ	Skrapar	11.7	7.1±0.02	61±0.7	Yes
VKKZH 2	Zarcin	12.5	6.1±0.6	145±0.7	Process
MRLT 4	Durres	12.4	5.2±0.03	181±0.7	Yes
MVRD 3	Narte	12.5	6.49±0.02	119±0.7	Yes
SHSHZ 2	Tirane	13.1	7.1±0.02	119±0.7	Yes
SHSHZ 3	Tirane	13.2	7.5±0.05	161±0.7	Yes

SHSHZ 4	Tirane	13.2	7.2±0.05	379±0.7	Process
SHSHZ 5	Tirane	13.2	7.8±0.05	259±0.7	Yes
SNVZ	Tepelene	12.5	5.3±0.02	271±0.7	Process
K-MK 1	Librazhd	12.5	7.2±0.05	139±0.7	Yes
MRLT 5	Gjirokaster	13.1	7.4±0.02	219±0.7	Process
K-MK 2	Librazhd	12.5	7.4±0/07	235±0.7	Process
KRTSH 2	Gjirokaster	12.5	9.6±0.07	123±0.7	Process
TMPR	Koplik	13.3	5.9±0.07	421±0.7	Yes
MRLT 6	Tirane	11.7	5.9±0.07	49±0.7	Porocess
KBRNT 2	Berat	12.5	5.9±0.07	181±0.7	Yes
SHSHZ 6	Berat	12.4	5.9±0.07	61±0.7	Yes
KBRNT 3	Berat	12.5	7.8±0.05	101±0.7	Yes
VKKZH 2	Tirane	13.1	6.5±0.02	61±0.7	Yes
PNR 2	Leskovik	13.2	7.7±0.02	121±0.7	Process
MRLT 7	Berat	13.2	6.7±0.03	61±0.7	Process

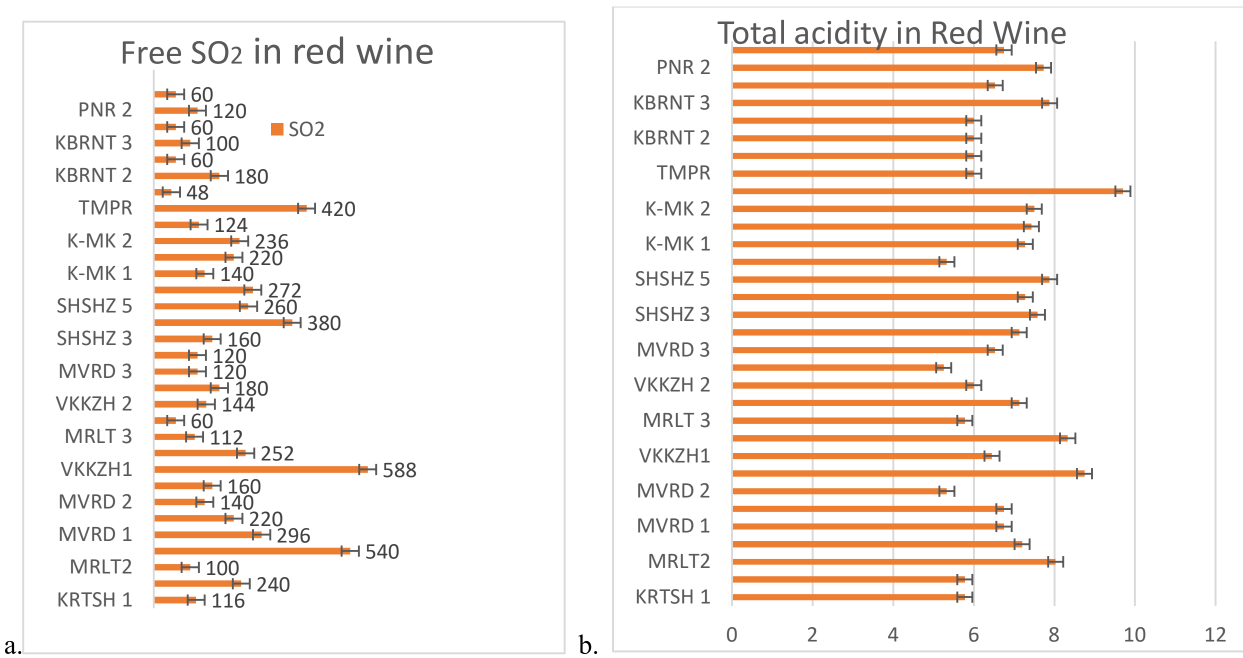


Figure 1. Contents of: a. total acidity and b. SO_2, for red wine

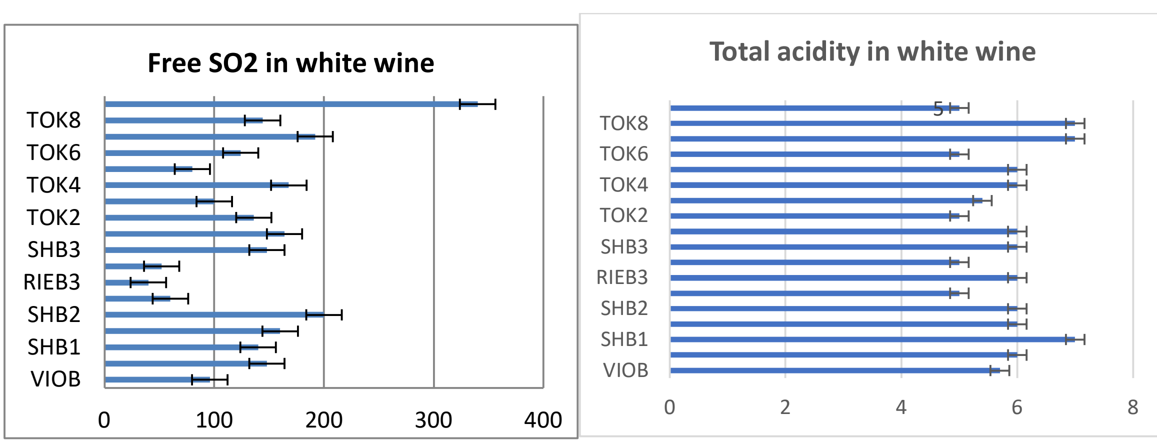


Figure 2. Contents of: a. total acidity and b. SO_2, for white wine

a. 

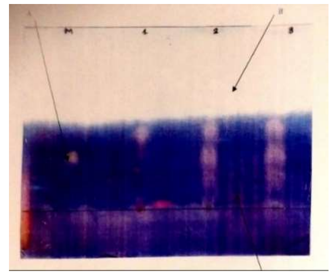
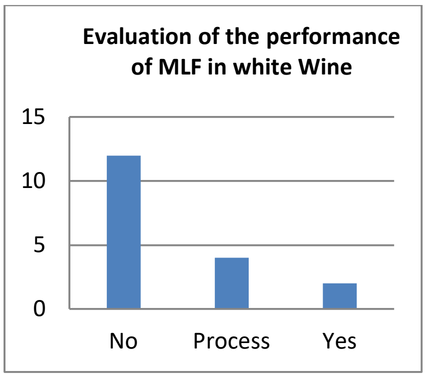
b. 

Figure 3. a.preparation of Watman paper No. 1 for the analysis of the determination of the presence of malic acid in wine (Drops of wine samples for analysis and Drops of the standard (malic acid) for comparison), b. the result on the dried paper after extraction (M- Malic Acid; A- Level of Malic Acid manifestation; B- level of lactic acid clearance

a. 

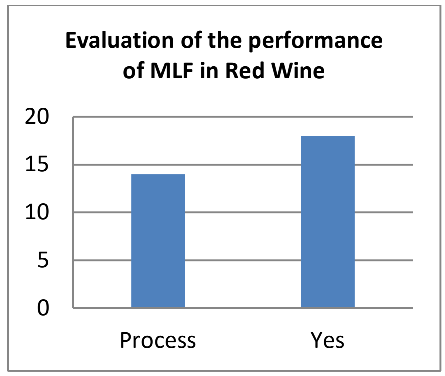
b. 

Figure 4. Evaluation of the performance of MLF in: a. white Wine and b. red wine

FML is important in terms of:bacterial stabilization: being a strong acid, malic acid is a substrate for several types of wild bacteria such as *Oenococcus, Pediococcus, Lactobacillus*, etc. Its consumption at this stage helps increase the stability of the wine by avoiding fermentation in the bottle, or further degradation of the wine. The formation of flavor elements: Malic acid gives the wine a strong taste, during FML this acid is dispersed, we have an increase in pH, the formation of lactic acid and a milder taste and dairy aroma.
Approximately, it is thought that the fermentation of 1g/l malic acid reduces the overall acidity by 0.6g/l. Malolactic fermentation is always accompanied by an increase in fluoric acidity. During malolactic fermentation, the formation of diacetyl occurs. Which is included in the aromatic complex obtained from malolactic fermentation. When this compound is present at a rate higher than 4mg/l, the aroma of butter dominates. Another transformation due to lactic acid bacteria is the decarboxylation of histidine, forming histamine, which is a toxic substance. This rare reaction is carried out only by some bacterial strains and under some conditions and is responsible for high amounts of histamine in 10mg/l wine. MLF is accompanied by a change in the color of the red wine, the intensity increases and the lively color becomes shaded.
During the action of *Leuconostoc oenos* bacteria to convert malic acid into lactic acid, this bacterium can transform citric acid into acetic acid (vinegar taste). Since MLF increases the pH, this can be a factor for the wine to be affected by other possible infections, therefore sulfiting should be carried out according to the normative. Partial or incomplete MLF can lead to gasification of the wine. Before bottling, a wine must have finished MLF or this fermentation must have finished.

4. Conclusions

In all cases of the production of high-quality red wines, malolactic fermentation is an important quality factor. From the results we can see a general acidity not lower than 4.5 gr/l tartaric acid, (specifically min 5.4 gr/l tartaric acid and max 8 gr/l tartaric acid, this especially in the samples that have not passed the MLF and in the white wine samples where the grapes were harvested before technical maturity) and free SO2 levels within the standard with the exception of one sample of white wine and 10 samples of red wine where in 4 of them it is 2 times higher, this is due to the indiscriminate treatment with SO2 that the producer did to preserve the wine from infection.

The assessment showed that only 40% have completed the FML, 36% are in the process, 24% have not yet started the FML. In some of them, fatty aromas and fermented vegetables were detected, which indicates the spontaneous development of the process, in 14% of them, the presence of diacetyl and high fluoric acidity was detected, which indicates that FML was not performed in the right time and manner.

From the organosensory evaluation, we note that: Wines that have undergone MLF are presented with body, with a rounded and lasting taste. The wines that are in the process present slightly vinegary and fatty aromas and a low level of gas, this is also due to the extended time of action of the lactic bacteria. Wines that have not started MLF are presented with strong acid taste and pronounced aromas of the grape variety used. In order to have the quality and durability of the wine, it is important that this process is carried out under control before the wine is bottled.

6. References

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