

RESEARCH ARTICLE

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The evaluation of spontaneous fermentation based on initial number of yeast in some autochthonous Albanian grape varieties

ROZETA HASALLIU^{1*}, KLOTILDA MARKU¹, KRENAR GOZHDARI¹, MARSEL KEÇI¹, RENATA KONGOLI¹¹Faculty of Biotechnology and Food, Agricultural University of Tirana

*Corresponding author; E-mail: rhasalliu@ubt.edu.al

Abstract

The aim of this study is to evaluate the spontaneous fermentation based on initial number of yeast in some autochthonous Albanian grape varieties. There are many microorganisms in grape that contribute to the production of wine. Some of them are wild yeast, lactic acid bacteria and acetic acid bacteria. The grapes contain indigenous yeasts which contribute in the spontaneous alcoholic fermentation during the vinification process. On the other hand we can have a controlled alcoholic fermentation by inoculating industrial yeast and controlling the fermentation temperature. In Albania, some of wine producers use spontaneous alcoholic fermentation and others inoculated yeasts that are *Saccharomyces cerevisiae*, *Saccharomyces bayanus* or a mix between two yeasts, *Saccharomyces cerevisiae* and *Saccharomyces bayanus*. During the 2017 year we have produced wine using some Albanian grape varieties like Kallmet, Shesh i Zi and Shesh i Bardhe which are obtained from different areas of Albania. In some grape varieties spontaneous fermentation couldn't start without the addition of *Saccharomyces cerevisiae* starter culture. In some others varieties the fermentation couldn't start even with the addition of starter culture. In the same grape variety Shesh i Bardhe obtained from different areas of Albania, in one vinification trial the fermentation didn't start even with the addition of starter culture. So, in this study we will do an assessment of initial number of indigenous yeasts in grape varieties used to do wine and a comparison of areas from which these varieties have been obtained.

Keywords: yeast; Albanian grape variety; wine; fermentation.

1. Introduction

There are many microorganisms in grape that contribute to the production of wine. Some of them are wild yeast, lactic acid bacteria and acetic acid bacteria. The grapes contain indigenous yeasts which contribute in the spontaneous alcoholic fermentation during the vinification process. On the other hand we can have a controlled alcoholic fermentation by inoculating industrial yeast and controlling the fermentation temperature [3,7,9,19]. Wine fermentations can be divided into two types: directly inoculated and uninoculated. Uninoculated fermentations are also called native flora or spontaneous or natural fermentation, and rely on the indigenous flora of the grapes and winery for fermentation. In Albania, some of wine producers use spontaneous alcoholic fermentation and others inoculated yeasts that are *Saccharomyces cerevisiae*, *Saccharomyces bayanus* or a mix between two yeasts, *Saccharomyces cerevisiae* and *Saccharomyces bayanus* [1,2,4,6,17]. Two principal species of *Saccharomyces* are found during alcoholic fermentation: *Saccharomyces cerevisiae* and *Saccharomyces bayanus*. *Saccharomyces bayanus* group includes strains that are able to ferment melibiose [5,8,9,10,18]. *Saccharomyces* is more commonly isolated from heavily damaged grapes. The presence of other yeast genera depends upon regional and climatic influences, the grape variety, disease pressure and level of damage of the grapes, and vineyard practices [11,20,22]. In addition to stage of ripening, many factors have been identified that impact the presence and numbers of yeasts on the surface of grapes. In general, the number of yeasts present on grapes increases with ripening. Seasonal variation has also been observed with warmer and dryer years yielding increased yeast populations [12,13,14,20]. *Saccharomyces bayanus* is yeast that starts the fermentation in low temperature. It produces glycerol in quantity more than *Saccharomyces cerevisiae* produces [15,16,21,23]. During the 2017 year we have produced wine using some Albanian grape varieties like Kallmet, Shesh i Zi and Shesh i Bardhe which are obtained from different areas of Albania.

In some grape varieties spontaneous fermentation couldn't start without the addition of *Saccharomyces cerevisiae* starter culture. In some others varieties the fermentation couldn't start even with the addition of starter culture. In the same grape variety Shesh i Bardhe obtained from different areas of Albania, in one vinification trial the fermentation didn't start even with the addition of starter culture. So, in this study we will do an assessment of initial number of indigenous yeasts in grape varieties used to do wine and a comparison of areas from which these varieties have been obtained. The aim of this study is to evaluate the spontaneous fermentation based on initial number of yeast in some autochthonous Albanian grape varieties.

2. Material and Methods

During the 2017 year we have produced wine using some Albanian grape varieties like Kallmet, Shesh i Zi and Shesh i Bardhe which are obtained from different areas of Albania. In some grape varieties spontaneous fermentation couldn't start without the addition of *Saccharomyces cerevisiae* starter culture. In some others varieties the fermentation couldn't start even with the addition of starter culture. For microbiological analysis PDA medium is prepared, sterilized in autoclave in 121°C for 15 minutes, and spread out in Petri dishes. 25 ml of grape juice from varieties of Kallmet, Shesh i Zi and Shesh i Bardhe are homogenized in 225 ml of peptone water. 5 tubes are filled with 9ml of peptone water and 1ml from the homogenized wine is putted in the first tube. 1 ml from the first tube is putted in the second tube. In this manner until in the 5-th tube and finally 5 dilutions are prepared, 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} . 1 ml from each tube is putted in Petri dishes with PDA medium. Petri dishes with PDA medium are incubated in thermostat at 25°C. After 48-72 hour Petri dishes are taken off from the thermostat and yeast colonies are counted.

3. Results and Discussion

In the table 1.1 are all the grape varieties taken for microbiological analyses and their origin of the area were these grapes are taken. The results from Shesh i Bardhe grape variety was 0 cfu/ml, means without yeast in the grape. Spontaneous fermentation cannot occur but also the inoculated fermentation with *Sacharomyces cerevisiae* didn't occur. This means that pesticides are used in the grape shortly before the harvest and it did not had the time to deplete his action. All the other grapes taken for microbiological analyses had the initial number of yeast in an amount that the spontaneous fermentation could occur.

Tabela 1. Grape varieties, origin and yeast's initial number.

Code	Grape Variety	Origin	Yeast used for fermentation	Date of Inoculation	Initial number of yeast (cfu/ml)
A20	Shesh i Bardhe	Koplik	<i>S. bayanus</i>	9/21/2017	0
A22	Kallmet	Koplik	<i>S. bayanus</i>	9/18/2017	3.3×10^6
A23	Kallmet	Koplik	<i>S. cerevisiae</i>	9/18/2017	2.9×10^6
A24	Kabernet	Koplik	<i>S. bayanus</i>	9/18/2017	2.2×10^6
A25	Kabernet	Koplik	<i>S. cerevisiae</i>	9/18/2017	1.4×10^6
A26	Ancelotti	Koplik	<i>S. bayanus</i>	9/18/2017	3.4×10^6
A27	Ancelotti	Koplik	<i>S. cerevisiae</i>	9/18/2017	6×10^5
B28	Kallmet	Rreshen	<i>S. cerevisiae</i>	9/19/2017	5×10^6
B29	Kallmet	Rreshen	<i>S. bayanus</i>	9/19/2017	4.4×10^5
B30	Shesh i Bardhe	Rreshen	<i>S. bayanus</i>	9/19/2017	4×10^4
B31	Shesh i Bardhe	Rreshen	<i>S. cerevisiae</i>	9/19/2017	2×10^4
B32	Shesh i Zi	Durres	<i>S. cerevisiae</i>	9/27/2017	1.4×10^6
S24	Shesh i Zi	Lunder	<i>S. cerevisiae</i>	9/12/2017	3.4×10^6
S25	Shesh i Zi	Lunder	<i>S. cerevisiae</i>	9/12/2017	3.6×10^6
S26	Shesh i Bardhe	Lunder	<i>S. bayanus</i>	9/15/2017	2.5×10^6
S27	Shesh i Bardhe	Lunder	<i>S. cerevisiae</i>	9/15/2017	1×10^6

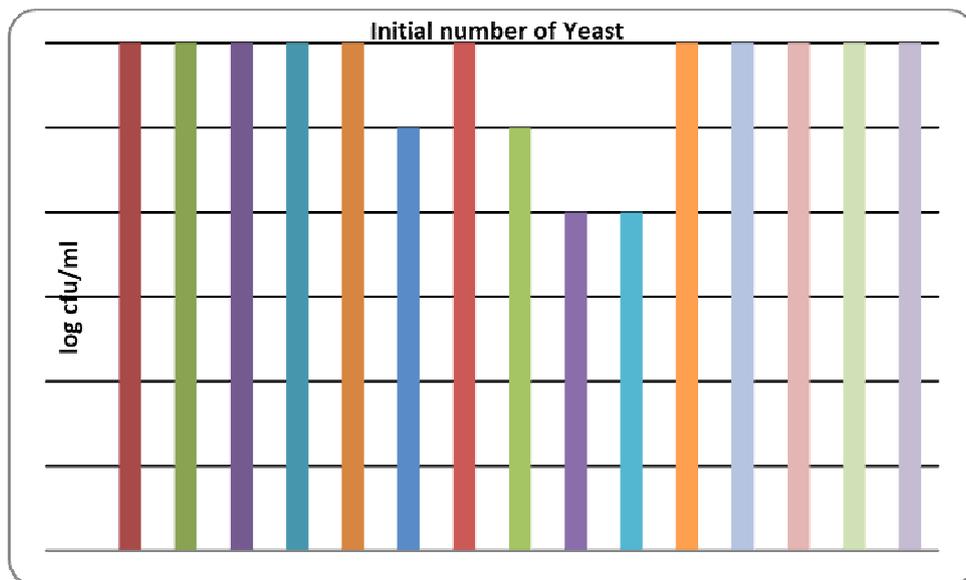


Figure 1. Initial number of yeast in grape varieties.

The initial number of yeasts in B30 and B31 varieties is lower than other grape varieties.

4. Conclusions

In conclusion we can say that use of pesticide just before harvest leads to a sterilization of the grape as shown from the results which would be a big problem in the case we want to conduct a spontaneous fermentation. All the other grape trials had enough yeast cells to start a spontaneous fermentation, with the exception of Shesh i Bardhe trials from Rreshen which had a quite lower cell count compared to the other trials.

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