Contributions concerning the study of the antiseptic effect of sulfur dioxide on the yeasts present in two wines provided by Cotnarivineyard

Carmen Daniela PETCU[1]; Constantin DELEANU[2]; Ioan MATEI[2]; Ştefan Dragoş COTAN[2]; Gabriel VIZITEU[2]; Viorel ANDRONIC[2]
[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Veterinary Medicine, 105 Independenţei Spl, District 5, 050097, Bucharest, Romania
[2]S.C. Cotnari S.A., Cotnari, 707120, Iași, Romania
e-mail: carmen28petcu@gmail.com

Abstract
Sulfur dioxide is one of the most used antiseptics in vinification. Its action on the "infection" microorganisms found in wine cellars is demonstrated by numerous studies. The present study shows that the antiseptic potential of sulfur dioxide is highly dependent on the concentration of its free form. The high concentrations of free sulfur dioxide corroborated with the prolonged contact period lead to the killing of the yeast cells quickly and evenly, while at low concentrations its lethal effect is greatly diminished. At low concentrations the lethal effect of sulfur dioxide is highly dependent on the contact time of the yeast cells with the environment. In this case, their mortality rate is very uneven. This phenomenon is also explained by the losses that occur due to the volatilization of sulfur dioxide, of oxidation or on account of its binding to other compounds.
Key words*: vinification, sulfur dioxide, yeast cells.*

Introduction

Although most strains of *Saccharomyces cerevisiae* synthesize sulfur dioxide during alcoholic fermentation, it does not play a significant role in reducing competing microbial populations, since once synthesized, it rapidly combines with other compounds thus losing its antiseptic role (King A.D., 1981; Ghimpeţeanu O.M. et al., 2013).

Therefore most of the sulfur dioxide used in vinification is of a technological nature (Jackson R.S., 2008; Ghimpeţeanu O.M. et al., 2018).

The forms, known in oenology under which sulfur dioxide is found in wines are: free sulfur dioxide and combined sulfur dioxide. From the sum of the free sulfur dioxide with the combined sulfur dioxide results the total sulfur dioxide (Cotea V.D., 1985; Cotea V.D. et al., 2009).

Free sulfur dioxide, directly titrated with iodine solution, represents a maximum of 30% of the total sulfur dioxide. It consists mainly of H_2SO_3, HSO_3, SO_3^{2-}.

The combined sulfur dioxide known in oenology also as bound sulfur dioxide results from reactions between sulfur dioxide and other substances. It has a very weak antiseptic effect, almost negligible (Cotea V.D. et al., 2009).

Materials and methods

Two different wines were studied, one of them being Grasă de Cotnari, the other Frâncușă (table 1), 2018 harvest, obtained from the experimental fermentation, spontaneous in glass vessels with the capacity of 50 liters. After the completion of the alcoholic fermentation and after decanting, the resulted wines had the following analytical parameters:

Table 1. The main parameters of Frâncușă and Grasă de Cotnari wines

Wine	Alcoholic strength (%v/v)	Glucose-fructose (g/l)	Total acidity (g/l tartaric acid)	pH	Free sulfur dioxide (mg/L)	Total sulfur dioxide (mg/L)
Frâncușă	12,5	2,1	7,4	3,2	2	6
Grasă de Cotnari	12,8	3,1	7,2	3,4	3	10

The methods used to determine the above mentioned parameters were:

Determination of alcoholic strength - method based on frequency oscillation OIV-MA-AS312-01A
Determination of glucose-fructose content - the enzymatic method OIV-MA-AS311-02
Determination of total acidity - potentiometric titration method OIV-MA-AS313-01
Determination of pH - potential difference method (pH electrode) OIV-MA-F1-06
Determination of sulfur dioxide - iodometric method OIV-MA-AS323-04B

In order to start the present study, each of the wines was transferred, using sterile equipment, in a glass container, previously sterilized, with the capacity of 50 liters. The number of viable yeast cells for each wine was then determined. The initial population of yeast was mainly made of *Saccharomyces spp.*, since the alcoholic fermentation of grape must is mainly completed by this genus (Irimia L.M. et al., 2018). Quantitatively, there were 2 x 105 cells *Saccharomyces spp.* viable/ml for Frâncușă wine and 3 x 104 cells *Saccharomyces spp.* viable/ml for Grasă de Cotnari wine.

To determine the number of viable cells, the study resorted to the usual analysis method, using a 40 X microscope lens and a Thoma cell counting chamber. Living and dead yeast cells were differentiated using methylene blue 0.01% solution (Painting K., Kirsop B., 1989). The taxonomic determination of the involved yeast was coordinated based on the classical methods of determination by analyzing the morphological and biochemical characteristics.

Then, according to the established protocol, from each of these containers, 6 samples of 1000 cm^3 each were taken, which were treated with 6% of liquid sulfur dioxide solution.

Each series of samples was treated as follows:

Frâncușă wine (initial yeast population 2 x 105 viable cells/ml)
1 sample was kept as a blank test, without administration of sulfur dioxide.
1 sample treated with sulfur dioxide, so that the amount of free sulfur boxid is 10 mg/ml.
1 sample treated with sulfur dioxide, so that the amount of free sulfur boxid is 30 mg/ml.
1 sample treated with sulfur dioxide, so that the amount of free sulfur boxid is 50 mg/ml.

Grasă de Cotnari wine (initial yeast population 3 x 104 viable cells/ml)
1 sample was kept as a blank test, without administration of sulfur dioxide.
1 sample treated with sulfur dioxide, so that the amount of free sulfur boxid is 10 mg/ml.
1 sample treated with sulfur dioxide, so that the amount of free sulfur boxid is 30 mg/ml.
1 sample treated with sulfur dioxide, so that the amount of free sulfur boxid is 50 mg/ml.

The samples were incubated at a constant temperature of 20°C, at time periods of 24, 48, 72, 96, 120 hours, cell viability determinations were performed for each of the samples separately.

Results and discussions

From the analysis of the collected data, in both cases, a clear antiseptic effect of sulfur dioxide is observed (table 2), an effect that varies directly proportional to the concentration and the contact period.

Looking at the initial number of yeast, for the two wines taken in the study, there is quite a significant difference. The difference is due to the fact that during fermentation, the processes start from different sugar quantities, Grasa de Cotnari accumulates a greater amount of sugars than Frâncuşa. In addition, it should be taken into account that the fermentation is spontaneous and there are two different strains of yeast, resulting in different fermentative capacities.

Table 2. Effect of sulfur dioxide concentration on cell viability - Frâncuşă wine

No.	Time (hours)	Number of living yeast cells (cells/ml)			
		Control sample	10 mg/l	30 mg/l	50 mg/l
1.	0	2×10^5	2×10^5	2×10^5	2×10^5
2.	24	2×10^5	$1{,}8 \times 10^5$	$1{,}4 \times 10^5$	3×10^4
3.	48	2×10^5	$1{,}2 \times 10^5$	1×10^5	5×10^3
4.	72	2×10^5	$8{,}4 \times 10^4$	$1{,}2 \times 10^3$	65
5.	96	2×10^5	$3{,}2 \times 10^3$	120	14
6.	120	2×10^5	86	4	1

In the case of Frâncuşă wine it was started from an initial number of 2 x 105 viable cells/ml. According to the recorded data, it is observed that the decrease of the cell viability is accelerated with the increase of the contact time of the cells with the antiseptic (sulfur dioxide), but also dependent on concentration. During 120 hours with increasing concentration of sulfur dioxide in the environment, a decrease in cell viability is observed reaching up to 1 cell/ml to 50 mg/l free sulfur dioxide (figure 1).

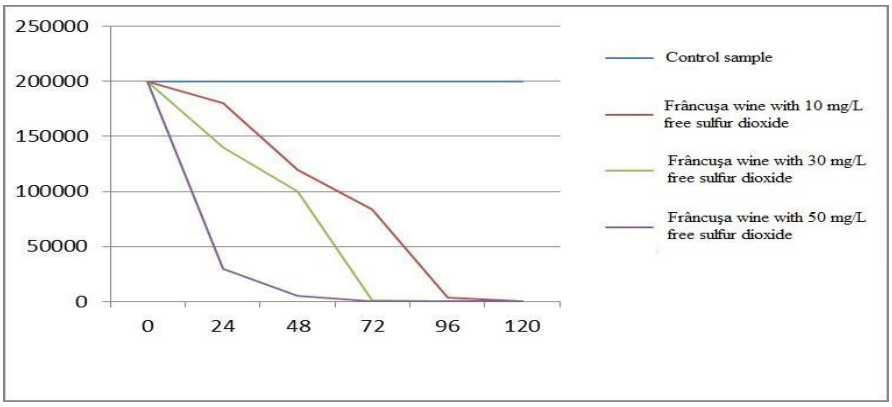

Figure 1 - Graphic representation of the effect of sulfur dioxide concentration in the environment on cell viability of Frâncuşă wine

In Grasă de Cotnari wine, although it started from a smaller number of viable yeast cells (3 x 104 living cells/ml), the effect of sulfur dioxide was the same, meaning that once the contact period and the concentration were increased, a significant decrease was observed in the number of viable cells (table 3, figure 2).

Table 3. Effect of sulfur dioxide concentration on cell viability - Grasă de Cotnari

No.	Time (hours)	Number of living yeast cells (cells/ml)			
		Control sample	10 mg/l	30 mg/l	50 mg/l
1.	0	3×10^4	3×10^4	3×10^4	3×10^4
2.	24	3×10^4	$2,6 \times 10^4$	$1,8 \times 10^4$	$1,2 \times 10^4$
3.	48	3×10^4	$1,3 \times 10^4$	8×10^3	3×10^3
4.	72	3×10^4	9×10^3	1×10^3	326
5.	96	3×10^4	500	118	35
6.	120	3×10^4	42	16	2

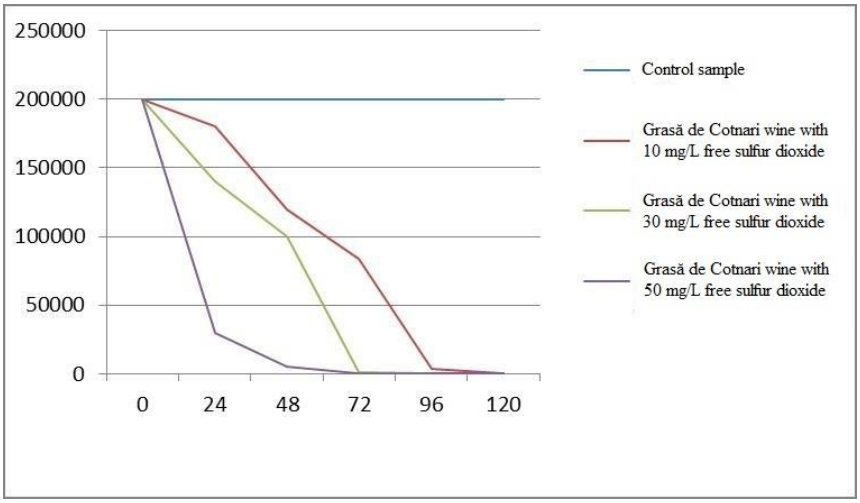

Figure 2 - Graphic representation of the effect of sulfur dioxide concentration in the environment on cell viability of Grasă de Cotnari wine

Comparative analysis, the yeast populations of the two wines have the same sensitivity to the effect of sulfur dioxide (figure 3, figure 4, figure 5).

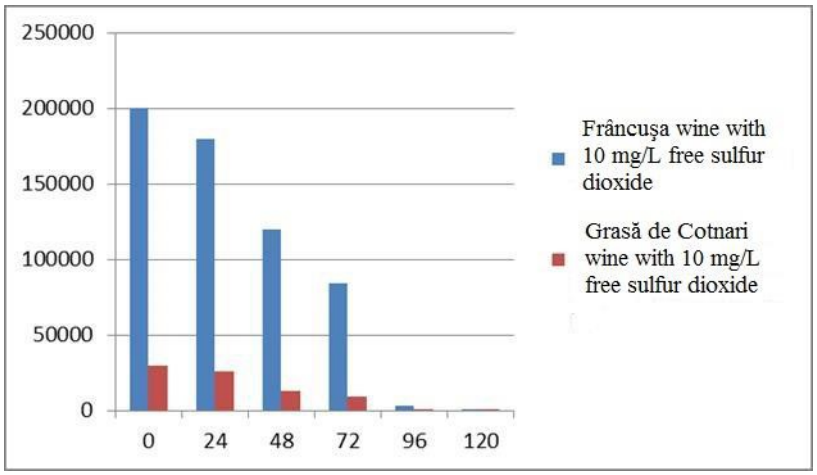

Figure 3 - Comparative graphical representation of the effect of sulfur dioxide at a dose of 10 mg/l free sulfur dioxide on Frâncuşa and Grasă de Cotnari wines

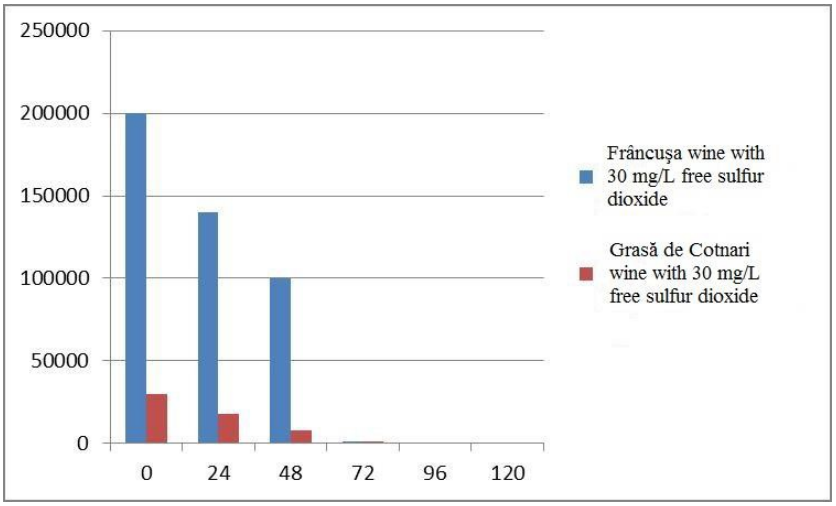

Figure 4 - Comparative graphical representation of the effect of sulfur dioxide at a dose of 30 mg/l free sulfur dioxide on Frâncuşa and Grasă de Cotnari wines

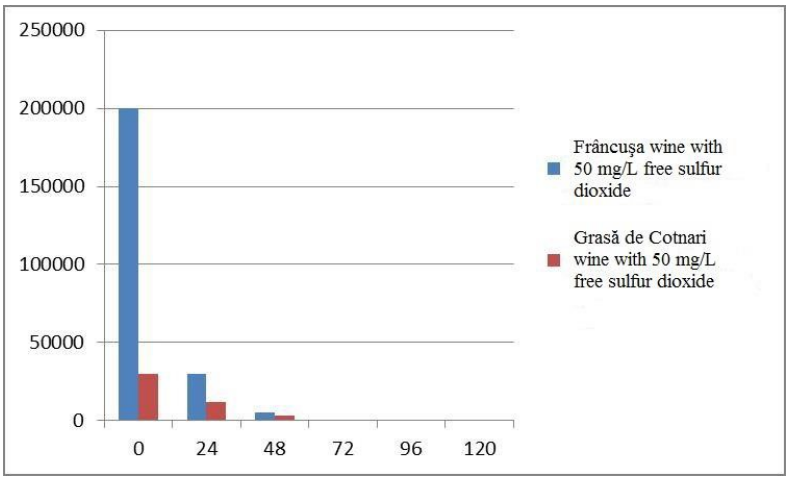

Figure 5 - Comparative graphical representation of the effect of sulfur dioxide at a dose of 50 mg/l free sulfur dioxide on Frâncușa and Grasă de Cotnari wines

Conclusions

The antiseptic effect of sulfur dioxide is clearly demonstrated in both wines analyzed.

As the contact period between the sulfur dioxide and the yeast cells increases, there is a drastic decrease in the number of living cells, a phenomenon observed in both wines.

At low concentrations, the lethal effect of sulfur dioxide is highly dependent on the contact time of the yeast cells with the environment. In this case, their mortality rate is uneven. This phenomenon is also explained by the losses that occur due to the volatilization of sulfur dioxide, of oxidation or on account of its binding to other compounds.

References

15. Cotea V.D., (1985). Tratat de oenologie, vol. 1, Editura Ceres, București.
16. Cotea V.D., Zănoagă C. V., Cotea V.V., (2009). Tratat de oenochimie, vol. 1, Editura Academiei Române, București.
17. Ghimpeţeanu O.M., Bocioacă E.I., Tăpăloagă D., (2013). Organoleptic, physical, chemical and microbiological assessment of different types of wines from a Romanian wine cellar. Scientific Works, C series, LIX(2):263.
18. Ghimpeţeanu O.M., Furnaris F., (2018). Assessment of white wines quality and hygiene parameters in a specialized unit. Lucrări Ştiinţifice Medicină Veterinară, Timişoara, LI(4):35-47.
19. Irimia L.M., Patriche C.V., Murariu O.C., (2018). The impact of climate change on viticultural potential and wine grape varieties of a temperate wine growing region. Applied Ecology and Environmental Research, 16(3):2663-2680.
20. Jackson R.S., (2008). Wine science, Principles and Applications, Academic Press Elsevier Inc.
21. King A.D., Ponting J.D., Sanshuck D.W., Jackson R., Mihara K., (1981). Factors Affecting Death of Yeast by Sulfur Dioxide. Journal of Food Protection, 44:92-97.
22. Painting K., Kirsop B., (1989). A quick method for estimating the percentage of viable cells in a yeast population, using methylene blue staining AFRC, Institute of Food Research, Norwich Laboratory, Colney Lane, Norwich NR4 7UA, United Kingdom.