# ABSTRACT

Wine is a living organism, being in a continuous evolution, but this particularity requires a permanent monitoring of all stages, starting from the harvesting of raw material - grapes - to the final product - wine. Consumer demand for high quality, nutritionally rich, health-conscious and safe food has increased, prompting food companies to adopt new food preservation techniques as modern alternatives to traditional ones.

The use of sulphur dioxide for preservation dates back to the end of the 18th century, mainly used in various food products, especially for the preservation of fruit juices and low *p*H drinks obtained by fermentation (Santos et al., 2012). Due to the fact that in some cases this preservative cannot fully ensure protection from a microbiological point of view, the use of antiseptics and antioxidants has been approved, which can complement the action of sulphur dioxide and prevent distortions caused by microorganisms or by existing or triggered chemical processes.

Research has demonstrated the contribution and positive impact of sulphur dioxide and dimethyldicarbonate treatments on the overall quality of wines and implicitly for the optimization of low sulphur dioxide winemaking technologies by Ough, 1975;1988; Lisanti and others, 2014; Santos and others, 2019; Lisanti and others, 2019; Yildirim and Darici, 2020; Muñoz García and others, 2021, etc.

The current trend among producers is towards food products obtained with reduced or no sulphur dioxide, so, the purpose of the two experiments was to monitor the evolution of wine quality following the administration of the treatments and to identify the effects produced by them. The novelty of these studies is to analyse the influence of different sulphur dioxide and dimethyldicarbonate treatment schemes on the micro-organisms deliberately introduced into the wines and the effects on their quality. In particular, the capacity of DMDC on the activity of yeasts inoculated in experimental wines and the identification of the changes caused by them was tested. The treatment schemes used for the design of the two studies were in accordance with the current legislation of the International Organisation of Vine and Wine.

The main objectives, which meet the mentioned purpose, are to implement the experimental protocols and to obtain variants for the two experiments, to influence the use of dimethyldicarbonate in wine production technology in order to reduce the amount of $SO_2$ and its ability to inhibit the growth of microorganisms, to study the influence of dimethyldicarbonate and $SO_2$ on physicochemical, chromatic and volatile compounds parameters, to monitor the antimicrobial efficacy of dimethyldicarbonate and sulphur dioxide by comparison with the results obtained in the literature and to monitor the aroma profile of the samples.

The experiments were carried out in the micro pilot station of the Oenology Laboratory of IULS (Ias University of Life Sciences). Forty-five samples were obtained for each experiment. The first experiment was carried out in 2018 processing grapes from the university's ampelographic collection of Muscat Ottonel and Fetească Regală varieties. The vinification process used was specific to white wines with the administration of antioxidant and antimicrobial protection treatments, namely sulphur dioxide (40, 80 160 mg/L) and dimethyldicarbonate (100, 200 mg/L) and yeast inoculum such as *Schizosaccharomyces pombe, Brettanomyces bruxellensis* in various concentrations (30/100 CFU/mL wine). For the second experiment carried out in 2020, a wine of

the Grüner Veltliner variety was used, which was treated in this case with 20, 40, 80 mg/L $SO_2$ and 100/200 mg/L DMDC and the yeast inoculum used was *Brettanomyces bruxellensis* and *Saccharomyces cerevisiae,* 30/100 CFU/mL wine. Samples obtained for both experiments were kept under optimal conditions until various laboratory analyses were performed.

In order to determine the physico-chemical analyses, the evolution of alcoholic concentration by simple distillation, volatile acidity and titratable acidity by titrimetry, pH by means of a pH meter, density by densimeter, free and total sulphur dioxide by iodometric method, sugars in must by refractometric method and in the case of wine by Luff Schrool method, malic and lactic acid were monitored. The evolution of chromatic parameters was carried out by UV-VIS spectrophotometry, volatile compounds by means of gascromatography and sensory analysis with the help of oenology specialists on the basis of tasting sheets. These determinations were carried out in the Oenology Laboratory of the Faculty of Horticulture of the "Ion Ionescu de la Brad" University of Life Sciences in Iasi according to the accredited norms and methods indicated by the legislation in force, as well as those reregulated by the OIV-International Organisation of Vine and Wine (OIV, 2020). Microbiological analyses were carried out in the Microbiology Laboratory of the USV Iasi. The determinations were carried out periodically, every three months, in triplicate or duplicate, for both experiments in order to monitor the evolution of the samples during storage and preservation.

Regarding the physico-chemical determinations for the variants of experiment I (V1-V45), it can be seen that there are no major differences between the variants studied, following the administration of the two treatments with sulphur dioxide and dimethyldicarbonate. However, the variants without DMDC treatment were found to be physicochemically unstable, with the values of the analysed parameters varying between the analysis periods. On the other hand, the V31-V45 variants, with the 160 mg/L $SO_2$ and DMDC treatment scheme, showed good stability, with similar values between the samples analysed.

A statistical analysis of the data shows that the PCA (principal component analysis) method can be applied to the results of the analysis of volatile compounds, thus revealing the correlations between the variables studied at a 95 % confidence interval. According to these data, high positive correlations were obtained between the identified compounds: ethyl lactate - ethyl acetate and 2-methyl-1-propanol - 1-propanol ($r > 0.8$). Positive correlations were also found between compounds such as: ethyl lactate - ethyl acetate; ethyl lactate - 1-butanol; isoamyl acetate - methanol; isoamyl acetate - 2-methyl-1-propanol; isoamyl acetate - 1-propanol, etc. Negative correlations were obtained between the pairs of compounds: ethyl lactate - acetaldehyde; ethyl acetate - acetaldehyde; 1-butanol - acetaldehyde.

The administration of the treatments proved to be optimal for the variants analysed, revealing concentrations of the volatile compounds of interest for this study (methanol and acetaldehyde) within the legislative limits and also stagnation of excessive growth of compounds that can impair the wines if their concentrations exceed the sensitive detection thresholds (ethyl acetate, isoamyl acetate, etc.). Thus, the effectiveness of the sulphur dioxide and dimethyldicarbonate treatments is demonstrated, which ensured the stability of the wines and inhibited the growth of pathogenic microorganisms.

Statistical analysis for the first set of determinations showed an increase in L* proportional to the level of sulphur dioxide administered for the first group of samples V1-V15. Regarding the

parameter **a***, in the case of the sulphur dioxide -40 samples with/without DMDC, the mean value showed the highest intensity, decreasing with increasing sulphur dioxide concentration. Thus, it can be seen that the wines have no specific colour, towards green, when the parameter "**-a***" should have negative values. The colour intensity varied according to the degree of oxidation, the lightest coloured variant being chromatically stable and the darkest coloured sample (almost brick coloured) being oxidised. The **b*** indicator also showed values inversely proportional to the level of sulphur dioxide administered. Thus, for the variant group sulphur dioxide-40 with/without DMDC, an average value was obtained, almost 4 times lower for the sulphur dioxide -80 samples with/without DMDC and about 6 times lower for sulphur dioxide-160 with/without DMDC.

As for the second set of analyses, samples V1-V15 are found to have a high degree of oxidation, with the wine colours having an oxidised hue, which shows that the 40 mg/L $SO_2$ treatment was insufficient and did not provide antioxidant protection over a longer period of time. For the colour indicator **L***, there was a considerable increase in value with increasing concentration of sulphur dioxide and DMDC administered (from 60 for the sulphur dioxide-40 variant set to 99 for sulphur dioxide-160). In the case of indicator **a***, the values obtained were much lower in samples treated with higher amounts of sulphur dioxide. This shows the good stability of the wines provided by the $SO_2$ and DMDC treatments. In the case of parameter **b***, the mean values decreased inversely with the level of sulphur dioxide administered. On the other hand, the indicators chroma, hue and intensity varied according to the dose of sulphur dioxide administered following the values obtained during the two analysis periods.

In this case, it can be observed that the best results were obtained for the variants treated with the maximum dose of 160 mg/L $SO_2$ and DMDC, as the proposed treatment scheme provided the wines with a stable colour and therefore antioxidant protection.

Comparing the results of the first set of analyses with those obtained in the second set, it can be seen that the 40 mg/L $SO_2$ and with/without DMDC samples did not exhibit chromatic stability, with V1-V15 showing a high degree of oxidation. Samples with 80 and 160 mg/L $SO_2$ and DMDC, respectively, are devoid of oxidation effects directly proportional to increasing $SO_2$ and DMDC doses.

Microbiological analyses of samples treated with 40 mg $SO/L_2$ with/without DMDC (V1-V15) showed no evidence of *Schizosaccharomyces pombe* and *Brettanomyces bruxellensis* yeasts, irrespective of the DMDC dose used, nor in samples where the treatment was not administered. However, species belonging to the lactic bacteria group were identified, with spherical (cocci) and cylindrical (bacilli) morphological forms, the wine showed deposit at the base of the bottle, colour changes (darker) and foamed strongly due to $CO_2$ accumulation. As for the samples treated with 80 mg/L $SO_2$ with/without DMDC, there was an improvement in the colour of the wines and a lower degree of refermentation, but also the absence of the yeasts mentioned for the samples treated with DMDC only. The best results can be attributed to the maximum concentrations of 160 mg/L $SO_2$ and DMDC, in this case the wines showed microbiological stability, high clarity and colour specific to the varieties from which they were obtained, free of oxidation and refermentation.

Following the application of the Anova One Way test, the sensory descriptors that showed significant differences (sig. < 0.05) for most of the variants analysed were highlighted, namely: vegetal character, aroma of ripe fruits, peaches, plums, green fruits, wild flowers, taste of marmalade, truffles, sensation of acid, bitter, salty, phenolic, unctuous, but also refermented and

oxidised character respectively. On the other hand, in relation to the treatment administered, exceptions were noted for the fruity notes of mango, grapefruit, green apple, honey, sweetness, unctuousness and oxidized character which did not show statistically significant differences. The best results were noted for the group of variants V31-V45 treated with 160 mg/L $SO_2$ and DMDC, the wines being the most appreciated, balanced in taste and aromas confirming also in this case the effectiveness of the treatments on the wines obtained. The results obtained are attributed to the potential of DMDC to improve and preserve the quality of the wine, to inhibit the development of pathogenic microorganisms and, above all, to maintain the aromatic profile of the wine.

Regarding the physico-chemical results of P1-P45 samples (experiment II), they confirm that the treatments with 20/40 mg/L $SO_2$ with/without DMDC vary from one treatment scheme to another, while those obtained following treatment with 80 mg/L $SO_2$ and DMDC provide physico-chemical stability between the parameters studied.

According to principal component analysis (PCA), average correlations ($r > 0.6$) were obtained between the groups of compounds: ethyl acetate - 2-methyl-1-propanol; ethyl acetate - isoamyl acetate; isoamyl acetate - acetaldehyde; 1-butanol - 1-propanol.

Negative correlations can be observed between the pairs of compounds: ethyl acetate-ethyl lactate; acetaldehyde-1-pentanol; methanol-isoamyl acetate, methanol-ethyl acetate, 1-pentanol-2-methyl-1-propanol, etc. Also, the arrangement of homogeneous groups in the factor axis system revealed a positive correlation between most of the experimental variants studied (e.g. P40-P42-P17-P34-P7-P1, P27-P36-P16-P45-P31-P19, P39-P13-P23-P12-P20, etc).

As for the chromatic parameters studied, the values of the **L*** indicator were increasing with increasing sulphur dioxide concentration with/without DMDC. The **a*** indicator was evident in samples P1-P15 with values ranging from -0.21 (control sample P1) to -0.54 (P12) with the negative sign representing the intensity of green colour. From a statistical point of view, the **b*** indicator, which suggests the intensity of the yellow shade, showed decreasing and significantly different values with the increase in the quantity of sulphur dioxide ($sig.<0.05$). These results show that in the wines obtained for the second experiment, during the increase in the dose of treatments, there was a noticeable improvement in colour, with the brightness reaching maximum values (100), an effect which can be attributed to the effectiveness of the treatments in maintaining and stabilising the colour of the wines.

In samples treated with 20, 40, 80 mg/L $SO_2$ with/without DMDC, differences can be observed in the evolution and activity of microorganisms during the storage of the wines. During the first 7 days of the experiment, inoculated yeasts were observed only in the control samples without DMDC, treated in this case with 20/40 mg/L $SO_2$. In contrast, in the 80 mg/L samples with/without DMDC no species were identified in the samples, the treatment having immediate action due to the synergistic effect with $SO_2$ being observed in this case. On the other hand, in terms of the following analysis steps, after 3 months, and after 6 months yeast multiplication can be observed in all samples treated with the lowest doses, i.e. 20/40 mg/L $SO_2$ but also with DMDC. On the other hand, samples treated with 80 mg/L $SO_2$ and DMDC showed the best stability in all analysis periods , yeasts were identified only in control samples without DMDC. Similar results were also obtained by Threlfall and Morris, 2002, with the effectiveness of the administered treatments on yeast concentrations being attributed to their synergy.

A sensory impact was confirmed by the treatments administered showing significant action on the sensory indicators analysed, except for vegetal, sweet and crunchy. Thus, positive correlations were obtained between pairs of descriptors: peach - ripe fruit, peach - grapefruit, ripe fruit - grapefruit, green fruit - wildflower, wildflower - vegetable, etc. Negative correlations were found between the indicators phenolic - ripe fruit, phenolic - peach, phenolic - grapefruit, salty - ripe fruit, salty - peach, salty - grapefruit, etc. Observing the distribution of the experimental variants, many positive correlations were found between samples: P10-P1-P13-P4-P11, P33-P32-P44, P21-P24-P30, P19-P28-P16, etc. On the other hand, negative correlations were obtained between samples P33-P1 or P42-P2. On the basis of these results, it can be seen that the synergistic action between sulphur dioxide and dimethyldicarbonate is evident and promising for further research as alternatives with optimal results for the microbiological and antioxidant stability of wines.

In conclusion, these results contribute to the optimization of strategies for obtaining wines with minimum concentrations of sulphur dioxide and with the addition of dimethyldicarbonate respectively, to improve the structure, chemical composition and implicitly the sensory characteristics.