Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Food Science and Technology
Open Access

The use of chitosan and an inactivated yeast to reduce the amount of sulfur dioxide in wine

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RESEARCH ARTICLE

Abstract

This study aims to evaluate the sulfur dioxide dynamics in two wines, dry white wine Fetească albă and dry red wine Fetească neagră made in Târnave vineyard, and to observe their evolution after application of Bactiless and Longevity to lower the level of SO_2. The wines from 2020 harvest were produced using traditional winemaking techniques. The experimental variants were: Control, Longevity (20, 30, 40, 0, and 40 g/hL) with Bactiless (20, 35, 50, 50 and 0 g/hL). For all the samples studied in this work, it was determined a decrease concentration of free and total sulfur dioxide. The best results obtained for the Fetească albă wine was the decrease of free SO_2 from 35.00 mg/L to 25.33±0.60 mg/L and total SO_2 from 120.50 mg/L to 117.50 mg/L by using 50 g/hL of Bactiless (CFAV4). For the Fetească neagră wine, the best results obtained were the decrease of free SO_2 from 32.50±0.29 mg/L to 14.83±0.18 mg/L and total SO_2 from 85.00±0.87 mg/L to 75.00±0.86 mg/L by adding 40 g/hL of Longevity and 50 g/hL of Bactiless (CFNV3). With an allowed SO_2 concentration, the evaluated wines maintained their varietal characteristics and organoleptic properties.

Keywords: bactiless, longevity, sulfur dioxide, wine

Received: 22 November 2023
Accepted: 13 April 2024
Published: 15 May 2024

DOI:
10.15835/buasvmcn-fst:2023.0053

INTRODUCTION

The enological business faces a complex challenge in producing wines without SO_2, because the free radicals released in the wine's oxidation reactions can cause significant deterioration, according to the production of oxidized substrates (Marchante et al., 2020[1]). Both antioxidant protection and microbiological stability can be achieved by adding SO_2 to wine (Lisanti et al., 2019). Despite these indisputable benefits, there are some potential negative clinical implications associated with SO_2 that have been raised due to its presence in wine, in a small population (about 1%) of „sulfite-sensitive" individuals (Lisanti et al., 2019) with reactions that include bronchospasm, bradycardia, gastrointestinal symptoms, as well urticaria, angioedema, hypotension, shock and in rare cases anaphylactic reactions (EFSA Scientific Opinion, 2014; Vally et al., 2009). Alternatives to SO_2 are preferred in order to limit the use of chemicals and increase winemaking stability (Valera et al., 2017) such as dimethyl dicarbonate, lysozyme, sorbic acid, chitosan, β-glucanases (Lisanti et al., 2019). One of the products approved by the European authority and used in this study is Bactiless chitosan, a natural non-allergenic biopolymer of fungal *Aspergillus niger* origin, used as a fining agent and antimicrobial in wines (Marchante et al., 2020[1]). The biopolymer is capable of forming compounds with iron and tartrate, reducing iron availability and inhibiting microbial growth (Nunes et al., 2016). Bactiless as a wine preservative has been shown to prevent chemical alterations as well as the appearance of spoiler yeasts and fungi (Chagas et al., 2012; Nardi et al., 2014; Petrova et al., 2016; Elmaci et al., 2015).

During the winemaking process, storage in the cellar and aging, the issue of wine oxidation is very important. To prevent wine oxidation, an inactivated dry yeast Longevity is used (Marchante et al., 2020[2]). It is a specific inactivated yeast with a high capacity for dissolved oxygen consumption (Marchante et al., 2020[2]). The studies demonstrate that the inactivated yeast Longevity consumes oxygen in a simulated medium at a rate similar to SO_2, indicating that it could be a good substitute to SO_2 and a way to reduce SO_2 content while still protecting wine from oxidation (Pones et al., 2019). Studies on the effect of two products, Bactiless and Longevity, on both white and red wines have been made (Marchante et al., 2020[1]; Marchante et al., 2020[2]; Marchante et al., 2020[3]; Valera et al., 2017; Pones et al., 2019) to show their ability to preserve wine and protect against oxidation.

A prestigious and significant viticultural area of Transylvania, Romania is Târnave vineyard (Chedea et al., 2021). This region is known for its high-quality white wines, which have a distinct flavor and good sugar/acidity balance (Iliescu et al., 2010; Cudur et al., 2014).

This study aims to evaluate the sulfur dioxide dynamics of two wines made in Tarnave Vineyard: a dry white wine (Fetească albă) and a dry red wine (Fetască neagră), to follow their development following the application of fungistatic and high in antioxidants substances, such as Bachiless chitosan and inactive dry yeast Longevity, to lower the SO_2 level.

MATERIALS AND METHODS

Chemicals

All used chemicals (0.02N iodine, sulfuric acid 1:3 - H_2SO_4, starch solution 1%, 1N potassium hydroxide - KOH, 96% alcohol, sodium hydroxide - NaOH, phenol red, phenolphthalein, tartaric acid- $C_4H_6O_6$) were purchased from Nordic Chemicals, Cluj-Napoca, Romania.

Pure-Lees™ Longevity (Bevitech SRL, Bucharest, Romania) is a specific inactivated yeast (*Saccharomyces cerevisiae*) developed by Lallemand Oenology located in Grenaa (Denmark), in collaboration with INRAE Montpellier (France) in order to provide a tool to help wine resist oxidation during storage and aging. Application is made in must/wine towards the end of alcoholic fermentation, with a sensory and color impact.

Bactiless™ (Bevitech SRL, Bucharest, Romania) is a 100% natural non-GMO (non-genetically modified organism) and non-allergenic biopolymer having as ingredients chitosan and chitin-glucan of fungal origin *Aspergillus niger*, which helps to control the bacteria population in wines, developed by Lallemand Oenology located in Grenaa (Denmark). His formula helps to lower the viable acetic and lactic bacteria population allowing easy removal. Despite its effectiveness towards a wide spectrum of bacteria, Bactiless does not affect yeast population. Its antibacterial effect can be enhanced with the use of SO_2, but it does not replace it, as doesn't have an antioxidant and antifungal effect. However, Bactiless can help to reduce the amount of SO_2 needed to control the lactic and acetic bacteria population (Valera et al., 2017; Elmaci et al., 2015).

Plant materials

In the period 12 to 23 September 2020, the grapes grown in the Târnave vineyard were harvested at optimal fruit maturity, with the appropriate health status for further winemaking processing.

Across approximately 2,250 km^2 of kilometers, the Târnave vineyard is the largest of all the vineyards in Transylvania, spanning across counties Alba, Mureș and Sibiu (Coros et al., 2019; Chedea et al., 2021), positioned at the intersection of the geographical coordinates of 46°- 47° Northern latitude and 23°-24° Eastern longitude, on the Transylvanian Plateau (Figure 1) (Iliescu et al., 2010; Călugăr et al., 2018; Cudur et al., 2014; Donici et al., 2019). Located on the southern slopes of the heights of this area, the vineyards start at an altitude of 250-270 m and up to 400-450 m, the slope of these lands being between 15-35% (Răcoare et al., 2022).

The climate characteristics of this area are moderate temperate-continental type, characterized by a lower level of heliothermal resources, very favorable for the cultivation of grapevines for wine grapes as the autumns are long, hot, and sunny, allowing the accumulation of sugars in the grapes, while the acidity remains quite high, favoring a good balance to obtain wines of high quality (POD) (Iliescu et al., 2010; Calugar et al., 2018; Donici et al., 2019; Chedea et al., 2021).

The temperature during flowering (17.0-17.8°C), the multiannual average temperature of July (19.6°C), the average maximum temperature in August (25.7°C), and the average atmospheric humidity from 1 pm of the same month (54%), indicates the presence of a cool and humid climate. Real insolation during the vegetation period is between 1130-1560 hours, with an average value of 1330 hours. During the vine vegetation period, an average of 440 mm of precipitation (360-500 mm) is recorded.

Most of the soils in the region are included in the class of argiluvisols, represented by brown, clay-illuvial brown, eumesobasic brown, and podzolite brown soils.

Fetească albă and Fetească neagră vines were grafted onto SO4-4 rootstocks, pruned with a Guyot (one arm) training system, and positioned vertically with eight shoots and two clusters each. Plantation management is conventional, with alternative grazing. Established in 2006, the Fetească albă plantation covers 1.8 hectares, with a planting distance of 2x1.2, 4166 vines per hectare, and a maximum fruit load of 40–42 buds. The plantation is 282 meters high and faces south, located at 46.1782° latitude and 23.8581° longitude. Located at 46.1028° latitude and 23.5102° longitude, the Fetească neagră plantation was established in 2010 and covers 0.12 hectares, with a planting distance of 2x1.0, 5000 vines per hectare, plateau with southern exposure at the base of a slope.

Figure 1. Large map of Romanian wine regions
(https://www.mapsland.com/europe/romania/large-map-of-romanian-wine-regions)

Six types of mechanical work were applied to the vineyards using different equipment, and these tasks were repeated based on the needs and specifications. These tasks included plowing on the second interval (March–April), applying herbicide under the row (April, July), milling on the plowed interval (April), cutting vegetation on the interval (May–September), performing phytosanitary treatments (May–September), and cutting and mechanically weeding (July–August).

Samples

Two wines from the year 2020, Fetească albă and Fetească neagră, were used in this study. The wines were obtained following the classical technology of the white wines making process (Coldea et al., 2014) and the red wine-making process (Bautista-Ortín et al., 2017). Shortly, grapes reception involved quantitative and qualitative analysis (health control, sugar content and total acidity). In order to obtain the must, grapes were destemmed and crushed, the white grapes were then subjected to pressing, skins and seeds were eliminated, and the must was left to ferment, at a maintained temperature of 10-12 °C. In the case of red grapes, following de-stemming and crushing, they were placed in the maturation - fermentation tank on the lees, where they spent seven days at a temperature of between 18 and 24 °C, after that they were pressed, and the must obtained was left for alcoholic fermentation, at 14-17 °C. The alcoholic fermentation in both cultivars was stopped by sulfitation with 1 mL SO_2/L wine, allowed to rest, racked in another tank, and bentonized with 100 g/hL bentonite. Before being filtered through porous cellulose filters, the wine that has been treated with bentonite is allowed to rest for a period of seven to twenty days (Sîrbu et al., 2022[2]). No additional yeast was added during the production of the wines under study, just those that were already present on the berry surface at harvest (Sîrbu et al., 2022[2]).

Following the vinification process, three experimental variants (Control, Variant 1 and Variant 2) of 50 L were made for each wine, namely ControlFA, V1FA, V2FA for Fetească albă wine and ControlFN, V1FN, V2FN for Fetească neagră wine (Figure 1). To see the effect of various SO_2 contents used to store white and red wines, different amounts of SO_2 and enzyme (lysozyme) were added to each variant (Table 1).

During the first 6 months, the SO_2 content was analyzed in several stages (yeast removal, 2nd pritoc, bentonization, and clarification, 6 months after filtration).

Table 1. Fetească albă and Fetească neagră cultivars from the 2020 harvest

Variants	Quantity (L)	SO_2 dose - 6% conc. (mg/L)	Enzyme dose (g/hL)
ControlFA	50	50	0
V1FA	50	30	25
V2FA	50	10	50
ControlFN	50	50	0
V1FN	50	30	25
V2FN	50	10	50

At the beginning of September 2021, 6 months after the first filtration the blending of the Control, Variant 1 and Variant 2 (CFA and CFN) was made (Figure 2), for which the following parameters were analyzed: free and total SO_2 (mg/L), alcohol content (%) and volatile acidity (g/L acetic acid - CH_3COOH). From the obtained coupage, 5 experimental variants and a control were made of 5 L each for Fetească albă (CFA, CFAV1, CFAV2, CFAV3, CFAV4, CFAV5) and 5 L for Fetească neagră (CFN, CFNV1, CFAV2, CFNV3, CFNV4, CFNV5), in which various amounts of Longevity and Bactiless are introduced (Table 2).

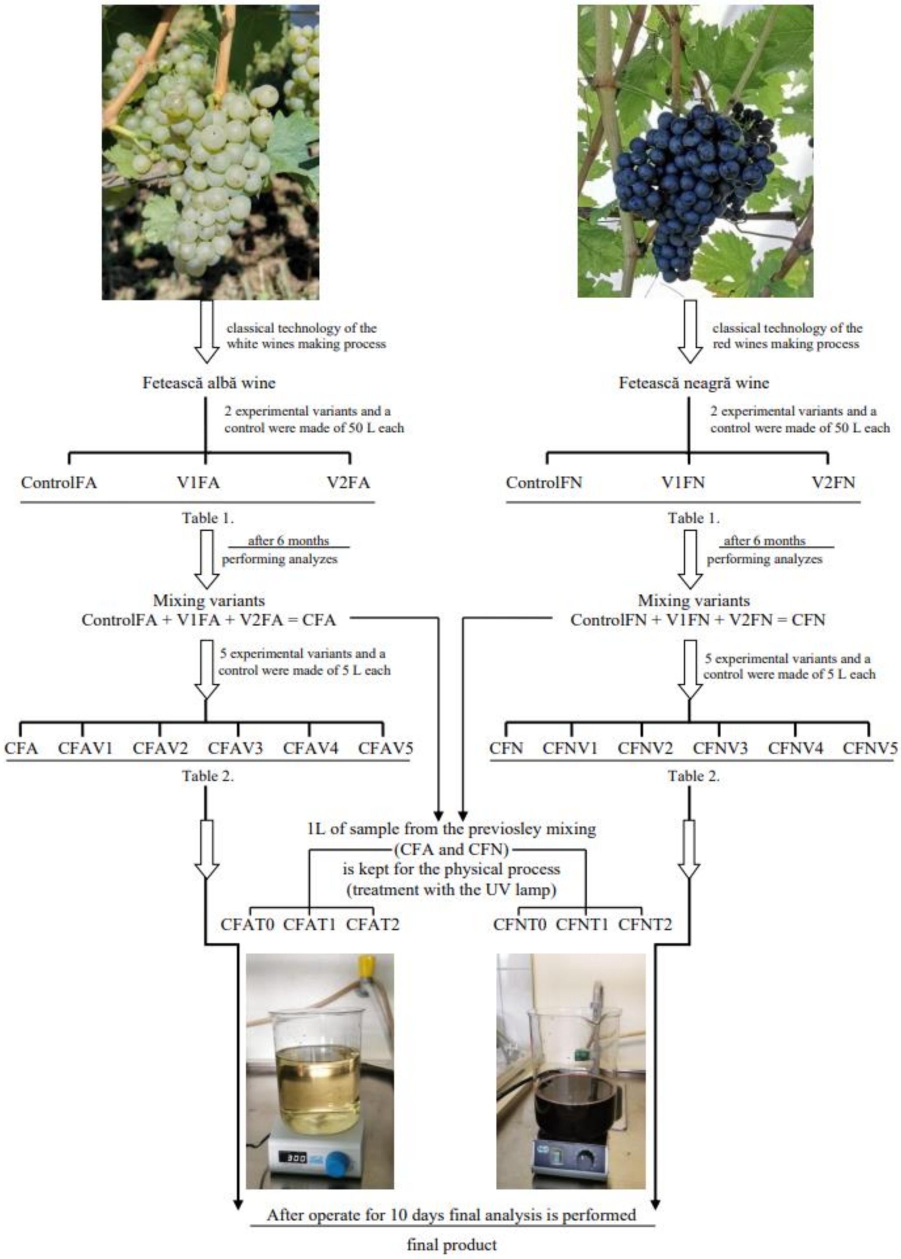


Figure 2. The total process of obtaining the two variants

Table 2. Experimental variants made after blending

Quantity (L)	Experimental variant		Product 1 - Longevity (g/hL)	Product 2 - Bactiless (g/hL)
5	CFA	CFN	0	0
5	CFAV1	CFNV1	20	20
5	CFAV2	CFNV2	30	35
5	CFAV3	CFNV3	40	50
5	CFAV4	CFNV4	0	50
5	CFAV5	CFNV5	40	0

The substances were left to operate for 10 days, then a general analysis of the composition was performed, by measuring the following parameters: free and total SO_2 (mg/L), alcohol content (%), total acidity (g/L tartaric acid – $C_4H_6O_6$), volatile acidity (g/L acetic acid - CH_3COOH), total sugars (g/L), and total dry extract (g/L).

One liter of sample from the previously obtained coupage is kept for the physical process, namely the treatment with the UV lamp, prior to the 5 variants being realized. For this treatment, the one-liter sample is placed in a glass vessel on a magnetic stirrer (Figure 1). The UV lamp is turned on and the shaker is placed under the hood. Three determinations are made throughout this period in three steps: T zero = after 1 minute, T1 = after 1 hour, and T2 = after 2 hours. After the irradiation process, the same determinations are made as after the blending process.

In Table 3, the samples taken in this study are described.

Table 3. Analyzed wine samples

Sample	Description
ControlFA	Fetească albă wine – Control variant
V1FA	Fetească albă wine – Variant 1
V2FA	Fetească albă wine – Variant 2
ControlFN	Fetească neagră wine – Control variant
V1FN	Fetească neagră wine – Variant 1
V2FN	Fetească neagră wine – Variant 2
CFA	Fetească albă wine – Coupage of ControlFA, V1FA and V2FA
CFAV1	Fetească albă wine – Coupage Variant 1
CFAV2	Fetească albă wine – Coupage Variant 2
CFAV3	Fetească albă wine – Coupage Variant 3
CFAV4	Fetească albă wine – Coupage Variant 4
CFAV5	Fetească albă wine – Coupage Variant 5
CFN	Fetească neagră wine – Coupage of ControlFN, V1FN and V2FN
CFNV1	Fetească neagră wine – Coupage Variant 1
CFNV2	Fetească neagră wine – Coupage Variant 2
CFNV3	Fetească neagră wine – Coupage Variant 3
CFNV4	Fetească neagră wine – Coupage Variant 4
CFNV5	Fetească neagră wine – Coupage Variant 5

Chemical analysis

Free and total sulfur dioxide content

Free and total sulfur dioxide (mg/L) it was determined using a modified iodometric method from ASRO-SR 6182-13:2009, through titration with iodine 0.02 N. 25 mL of wine sample, 2.5 mL of 1:3 H_2SO_4, and 1 mL of 1% starch solution have been used for the determination of free SO_2, and then titrated with iodine until the color changed. For the determination of the total SO_2, 25 mL of the sample was added over 12.5 mL of KOH and allowed to react for 15 minutes. After that, 5 mL of H_2SO_4 and 1 mL of starch solution were added. Titration with iodine was then performed until the color changed (OIV-MA-AS323-04B).

Alcohol content

Following the manufacturer's instructions, the alcohol content (% vol) was measured using a Dujardin-Salleron electric ebulliometer and a 10% standard solution (v/v), consisting of 96% (v/v) alcohol and distilled water (https://www.dujardin-salleron.com/documents/fiches/589b20d4a2332--160350---ft-ebulliometer-en.pdf) (Sîrbu et al., 2022[2]).

Total acidity measurement

Total acidity, expressed in g/L tartaric acid ($C_4H_6O_6$) was determined by the titration method. A mixture of 10 mL of wine sample and 10 mL of distilled water was titrated with 0.1N sodium hydroxide in the presence of the phenol red indicator, following the change of the sample color. After adding the sodium hydroxide solution, the titration is continued until the indicator becomes orange, according to OIV-MA-AS313-01: R2015.

Volatile acidity measurement

Volatile acidity, expressed in g/L acetic acid (CH_3COOH) was determined by the distillation method, using a solution of 0.1 N sodium hydroxide to titrate the wine the presence of phenolphthalein as indicator, and steam distillation to separate the volatile acids from the wine. The sample is acidified with tartaric acid to release the salts of volatile acids from the wine before they are entrained by water vapor, according to OIV-MA-AS313-02: R2015.

The total dry extract content

The total dry extract (g/L) is determined indirectly, based on the relative density of dealcoholised wine. The unit of measurement for extract is the amount of sucrose dissolved in 1 liter of water to create the same density as the alcohol-infused wine (OIV-MA-AS2-03B: R2012, 2012; Sîrbu et al., 2022[2]).

Total sugars content

Total sugars (g/L) are determined by their reducing action on an alkaline solution of a copper salt, according to method OIV-MA-AS311-01A (2009). Clarification of the wine using neutral lead acetate is the first step. Then, 25 mL of the alkaline copper salt solution, 15 mL of water, 10 mL of the clarified solution, and a few small pieces of pumice stone were added to the mixture. Using a reflux condenser, the mixture reaches the boiling point in 2 minutes and is kept boiling for exactly 10 minutes. Titration with a 0.1 M sodium thiosulfate solution is then performed, and the results are expressed. The amount of sugar, expressed as invert sugar, contained in the test sample is given in a table by number (n '- n) of mL of sodium use thiosulfate (OIV-MA-AS311-01A R2009; Sîrbu et al., 2022[2]).

Statistical analysis

The experimental data were made in duplicate and analyzed with the program Statview 5.0 performing one-way analysis of variance (ANOVA), followed by a Fisher protected least significant difference (PSLD) test. P values lower than 0.05 were considered significant while *p* values between 0.05 and 0.1 were considered as tendencies.

RESULTS AND DISCUSSIONS

The obtained results are structured and presented in the following tables and figures. Table 4 displays the values of the parameters analyzed for a period of 6 months from the obtaining of the first variants for both cultivars, followed by Table 5 with the results obtained after the blending of the variants.

Table 4. Evolution of free and total SO_2

Experimental variant	Removing yeast		2nd pritoc/ decanting		After treatment with bentonite and clarifying		Summary of organoleptic qualities	6 months after filtration	
	Free SO_2 (max 50.0 mg/L)*	**Total SO_2** (max 200.0 mg/L)*	**Free SO_2** (max 50.0 mg/L)*	**Total SO_2** (max 200.0 mg/L)*	**Free SO_2** (max 50.0 mg/L)*	**Total SO_2** (max 200.0 mg/L)*		**Free SO_2** (max 50.0 mg/L)*	**Total SO_2** (max 200.0 mg/L)*
ControlFA	12.5	77.5	10.0	72.5	32.5	117.5	with freshness	37.6	120.0
V1FA	17.5	77.5	15.0	75.0	32.5	115.0	with freshness	35.0	127.5
V2FA	20.0	75.0	17.5	75.0	22.5	92.5	with freshness	25.0	100.0
ControlFN	25.0	70.0	20.0	60.0	25.0	60.0	freshness and fruitfulness	25.0	85.0
V1FN	27.5	72.5	20.0	60.0	12.5	60.0	freshness and fruitfulness	22.5	87.5
V2FN	22.5	62.5	17.5	50.0	20.5	57.0	freshness and fruitfulness	25.0	65.0

Note: * the maximal and minimal values were reported as published by Ţârdea (2007)

The evolution of free and total sulfur dioxide is observed in Table 4, which indicates an increase until the moment of storage when the values remain below the imposed limit for almost all variants of both cultivars.

Table 5. Analysis of variants after blending

Experimental variant	Free SO_2 (max 50.0 mg/L) *	Total SO_2 (max 200.0 mg/L) *	Alcohol (min 8.00-max 14.20 % vol) **	Volatile acidity (min 0.08-max 1.10 g/L CH_3COOH) **
CFA	35.00	120.50	5.59	0.22
CFN	32.50	85.00	6.53	0.22

Note: * the maximal and minimal values were reported as published by Ţârdea (2007); **the maximal and minimal values were reported as published by Er and Atasoy (2016)

After blending, from the ControFA, V1FA and V2FA variants, the CFA variant was obtained for the Fetească albă cultivar, and from the ControlFN, V1FN and V2FN variants, the CFN variant was obtained for the Fetească neagră cultivar, with values of free SO_2 up to 35 mg/L and total SO_2 up to 120.50 mg/L (Table 5). Before being subjected to treatment with Longevity and Bactiless, 1 liter of each of the two variants formed was retained to carry out the physical procedure described in Table 6.

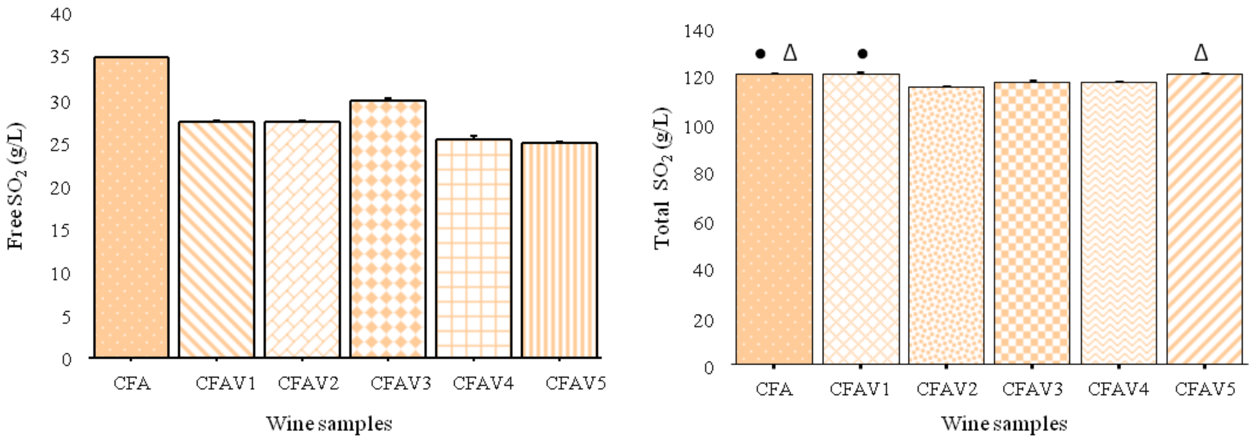
Table 6. Physical procedure (UV lamp treatment)

Variants	Period of time	Free SO_2 (max 50.0 mg/L) *	Total SO_2 (max 200.0 mg/L) *	Alcohol (min 8.00-max 14.20 % vol) **	Volatile acidity (min 0.08-max 1.10 g/L CH_3COOH) **
CFAT0	after 1 min	35.00	120.50	5.59	0.22
CFAT1	after 1 hour	25.00	105.00	5.58	0.22
CFAT2	after 2 hours	20.00	105.00	5.58	0.22
CFNT0	after 1 min	32.50	85.00	6.53	0.22
CFNT1	after 1 hour	22.50	77.50	6.53	0.22
CFNT2	after 2 hours	20.00	72.50	6.53	0.22

Note: T0=after 1 minute, T1= after 1 hour, T2= after 2 hours; ** the maximal and minimal values were reported as published by Ţârdea (2007) **the maximal and minimal values were reported as published by Er and Atasoy (2016)

After the physical procedure, free and total SO_2 (mg/L), alcohol content (%), and volatile acidity (g/L CH_3COOH) were determined for the samples CFAT0, CFAT1, CFAT2 of the Fetească albă cultivar and CFNT0, CFNT1, CFNT2 samples of the Fetească neagră cultivar (Table 6). The best results for free and total SO_2 (mg/L) were obtained after T2=after 2 hours, for both cultivars. After the two-hour process, the white cultivar recorded a decrease in the concentration of free SO_2 of 15 mg/L and 15.50 mg/L in the total SO_2 concentration. For the red cultivar, the decreases were 12.50 mg/L of the free SO_2 concentration and 12.50 mg/L of the total SO_2 concentration. For both cultivars, the volatile acidity remained unchanged, as well as the amount of alcohol, with a slight decrease for the white cultivar (CFAT1, CFAT2).

For the samples studied in this work, Figures 3(a) and 4(a) show the decreasing concentration of free SO_2 as determined for the five variants of each cultivar at the end of the performed treatments. The total SO_2 decreases, presented in Figures 3(b) and 4(b), are far less significant, compared to free SO_2.

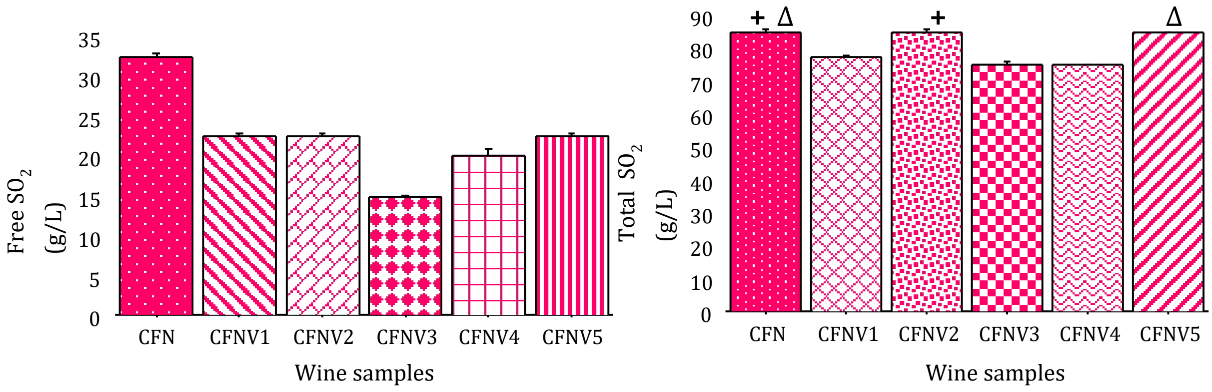


(a) (b)

Figure 3. (a) Free sulfur dioxide in Fetească albă wine samples; (b) Total sulfur dioxide in Fetească albă wine samples. The differences between the samples from a graphic are statistically different ($p < 0.05$) excepting the variants with the same letter which are statistically non-significant ($p > 0.05$). • - CFAV1 statistically non-significant when compared with CFA, Δ - CFAV5 statistically non-significant when compared with CFA.

Free SO_2 concentrations for the Fetească albă cultivar decreased in the following order CFA (35.00 ± 0.01 mg/L) > CFAV3 (30.00 ± 0.29 mg/L) > CFAV1(27.50 ± 0.29 mg/L) = CFAV2 (27.50 ± 0.29 mg/L) > CFAV4 (25.33 ± 0.60 mg/L) > CFAV5 (25.00 ± 0.17 mg/L), with statistically significant differences between CFA and CFAV5, closely followed by CFAV4.

Regarding the total SO_2 concentration for the same cultivar, the decrease occurred in the following order CFA (120.50 ± 0.01 mg/L) > CFAV1 (120.00 ± 0.87 mg/L) = CFAV5 (120.00 ± 0.29 mg/L) > CFAV3 (117.50 ± 0.29 mg/L) = CFAV4 (117.50 ± 0.01 mg/L) > CFAV2 (115.00 ± 0.40 mg/L), with statistically significant differences between CFA and CFAV2.



(a) (b)

Figure 4. (a) Free sulfur dioxide in Fetească neagră wine samples; (b) Total sulfur dioxide in Fetească neagră wine samples. The differences between the samples from a graphic are statistically different ($p < 0.05$) except the variants with the same letter which are statistically non-significant ($p > 0.05$). + - CFNV2 statistically non-significant when compared with CFN, Δ - CFNV5 statistically non-significant when compared with CFN.

The concentration of free SO_2 decreases for the Fetească neagră variety in the following order: CFN (32.50 ± 0.29 mg/L) > CFNV1 (22.50 ± 0.29 mg/L) = CFNV2 (22.50 ± 0.29 mg/L) = CFNV5 (22.50 ± 0.29 mg/L) > CFNV4 (20.00 ± 0.87 mg/L) > CFNV3 (14.83 ± 0.18 mg/L), with statistically significant differences between CFN and CFNV3.

With values not very high and close to each other, the concentration of total SO_2 decreases for the variants of the red cultivar as follows: CFN (85.00 ± 0.87 mg/L) = CFNV2 (85.00 ± 0.58 mg/L) = CFNV5 (85.00 ± 0.01 mg/L) > CFNV1 (77.50 ± 0.29 mg/L) > CFNV3 (75.00 ± 0.86 mg/L) = CFNV4 (75.00 ± 0.01 mg/L).

Once the substances operate for 10 days, the following parameters were assessed: alcohol content (%), total acidity (g/L $C_4H_6O_6$), volatile acidity (g/L CH_3COOH), total sugars (g/L) and total dry extract (g/L), as presented in Table 7.

Table 7. Analytical values of the wines after 10 days from the addition of Longevity and Bactiless

Experimental variant	**Alcohol** (min 8.00-max 14.20 % vol) **	**Total acidity** (min 3.80-max 14.20 g/L $C_4H_6O_6$) *	**Volatile acidity** (min 0.08-max 1.10 g/L CH_3COOH) **	**Total sugars** (max 5.0g/L) *	**Total dry extract** (min 21.00 g/L) *
CFA	12.50	5.54	0.20	1.92	20.70
CFAV1	12.40	5.49	0.21	1.80	19.90
CFAV2	12.50	5.42	0.19	1.80	21.80
CFAV3	12.50	5.42	0.24	1.92	23.80
CFAV4	12.60	5.49	0.23	1.80	19.80
CFAV5	12.50	5.42	0.22	1.80	20.00
CFN	12.50	6.53	0.33	2.04	26.70
CFNV1	12.60	6.53	0.36	2.16	25.20
CFNV2	12.50	6.46	0.32	2.16	25.30
CFNV3	12.50	6.46	0.33	2.04	25.40
CFNV4	12.60	6.46	0.32	2.28	25.50
CFNV5	12.50	6.46	0.38	2.04	25.20

Note: * the maximal and minimal values were reported as published by Ţârdea (2007); **the maximal and minimal values were reported as published by Er and Atasoy (2016)

The alcohol content of the analyzed wines recorded the highest value for the CFAV4 sample of the white cultivar and for samples CFNV1, CFNV4 of the red cultivar, with 12.60 % vol., not far from the minimum value obtained. The lowest value of alcohol it was for the CFAV1 sample of the white cultivar, with 12.40 % vol., respectively 12.50 % vol. for the CFN, CFAV2, CFNV3 and CFNV5 samples of the red cultivar. Values for total acidity were between 5.42 g/L $C_4H_6O_6$ (CFAV2, CFAV3, CFAV5) and 5.54 g/L $C_4H_6O_6$ (CFA) for the Fetească albă cultivar, and between 6.46 g/L $C_4H_6O_6$ (CFNV2, CFNV3, CFNV4, CFNV5) and 6.53 g/L $C_4H_6O_6$ for the Fetească neagră cultivar. Volatile acidity had values between 0.19 g/L CH_3COOH and 0.38 g/L CH_3COOH, with the lowest values for the Fetească albă cultivar and highest for the Fetească neagră cultivar. In all studied wines, the total sugar levels were within limits imposed for the high-quality wines from the Târnave vineyard (max. 5.0 g/L), with the lowest value of 1.80 g/L (CFAV1, CFAV2, CFAV4, CFAV5) for the samples of the Fetească albă cultivar, and with the highest value of 2.28 g/L (CFANV4) for the sample of the Fetească albă cultivar. Given the values observed in the analyzed samples within this study, it can be concluded that the findings align with the established values reported by Er and Atasoy (2016) and Ţârdea (2007).

Table 8 presents the sensorial characteristics of the analyzed wines.

Table 8. Sensorial analysis of Fetească albă and Fetească neagră wines

Cultivar	Harvest year	Appearance	Colour	Aroma/ Bouquet	Taste	Acidity
CFA	2020	excellent limpidity	yellow	pleasant, specific	pleasant, dry, with freshness	medium
CFAV1	2020	excellent limpidity	yellow	pleasant, specific	pleasant, dry, with freshness	medium
CFAV2	2020	excellent limpidity	white yellow	pleasant, specific	pleasant, dry, with freshness	medium
CFAV3	2020	excellent limpidity	yellow	pleasant, specific	pleasant, dry, with freshness	medium
CFAV4	2020	excellent limpidity	white yellow	pleasant, specific	pleasant, dry, with freshness	medium
CFAV5	2020	excellent limpidity	white yellow	pleasant, specific	pleasant, dry, with freshness	medium
CFN	2020	excellent limpidity	ruby	pleasant, specific	pleasant, dry, with freshness and fruitfulness	medium
CFNV1	2020	excellent limpidity	ruby	pleasant, specific	pleasant, dry, with freshness and fruitfulness	medium
CFNV2	2020	excellent limpidity	ruby	pleasant, specific	pleasant, dry, with freshness and fruitfulness	medium
CFNV3	2020	excellent limpidity	ruby	pleasant, specific	pleasant, dry, with freshness and fruitfulness	medium
CFNV4	2020	excellent limpidity	ruby	pleasant, specific	pleasant, dry, with freshness and fruitfulness	medium
CFNV5	2020	excellent limpidity	ruby	pleasant, specific	pleasant, dry, with freshness and fruitfulness	medium

The sensory analysis presented in Table 8 shows that all wines have an excellent limpidity, a yellow (CFA, CFAV1, CFAV3) or white-yellow color (CFAV2, CFAV4, CFAV5) for Fetească albă wines and a ruby color for all Fetească neagră wines, and a pleasant specific aroma. All the wines are pleasant, with a dry taste, with freshness, and medium acidity (Table 8).

Other researchers have reported the advantages of using the two products Bactiless and Longevity. Nunes et al. (2016) describe Bactiless as a biopolymer that prevents the growth of microorganisms, used to produce white wines without the addition of sulphur dioxide as a preservative, and Valera et al. (2016) describe it as being at least as effective as SO_2 in preserving wine. As for Longevity, it is named by Pones et al. (2019) as the equivalent of SO_2 at its usual levels of use in both red and white wines. Their results demonstrate that Longevity consumes oxygen in a model medium at a rate similar to that of sulfur dioxide. As a result, it may be a good substitute for SO_2 and a way to use less of it while still protecting wine from oxidation.

Merchante et al. (2020) claim that Bactiless and Longevity may be taken into consideration as viable natural alternatives for reducing SO_2 in the production of red wines because this permits the development of red wines with phenolic potential, and their effect is greater if it is added in the pre-fermentative stage.

For free sulfur dioxide dosages from this study, the limits were between 25.00 ± 0.17 mg/l and 35.00 ± 0.01 mg/L for the white cultivar, 17.83 ± 0.18 mg/L and 32.50 ± 0.29 mg/L for the red cultivar. The values for total sulfur dioxide were between 115.00 ± 0.40 mg/L and 120.50 ± 0.01 mg/L for the white cultivar, 75.00 ± 0.86 mg/L and 85.00 ± 0.87 mg/L for the red cultivar.

For the samples studied in this work, the best results obtained for the Fetească albă cultivar was the decrease of free sulfur dioxide from 35.00 ± 0.01 mg/L to 25.33 ± 0.60 mg/L and total sulfur dioxide from 120.50 ± 0.01 mg/L to 117.50 ± 0.01 mg/L by using 50 g/hL of Bactiless (CFAV4). The best results obtained for the Fetească neagră cultivar was the decrease of free sulfur dioxide from 32.50 ± 0.29 mg/L to 14.83 ± 0.18 mg/L and total sulfur dioxide from 85.00 ± 0.87 mg/L to 75.00 ± 0.86 mg/L by adding 40 g/hL of Longevity and 50 g/hL of Bactiless (CFNV3).

Every study result demonstrates the efficacy of Longevity and Bactiless products. When mixed or added individually, the products exhibit varying degrees of effectiveness.

In order to ensure both the control of microbiological activity and the protection against oxidations, the challenge for wine research is thus the development of strategies consisting of an integrated use of different methodologies with reduced doses of SO_2. For these two products to be used efficiently, they need the complement of SO_2. The administered doses must be carefully calculated, in order not to affect the quality of the wine or the color of the red wines, in the case of the Bactiless product. The cost of the finished product may increase since the costs of procurement and administration may be more expensive than with sulfur. But from an organoleptic point of view, it differs from sulfur due to the lack of production of a strong smell when there is an excess of the product.

CONCLUSIONS

The results show that the sulfur dioxide content of the two varieties of wines under study, Fetească albă and Fetească neagră, is below the legal limits allowed by the in-force legislation (free SO_2 at 50.00 mg/L and total SO_2 at 200.00 mg/L). In addition to various amounts of the two products Bactiless (20-50 g/hL) and Longevity (20-40 g/hL), this study demonstrated the usefulness of moderate SO_2 additions (10-50 mg/L SO_2 additions to must) in preserving volatile and non-volatile compounds in wine. For the samples studied in this work, it was observed a decrease in concentration of free and total sulfur dioxide. All the analyzed wines kept their varietal characteristics and organoleptic properties, such that they are high-quality white and red wines, with an admitted sulfur dioxide content.

Author Contributions: A.D.S Wrote the paper, performed the analyses, collected the data; V.S.C. Wrinting, supervision and project administration; L.L.T. Visualisation and supervision; M.C. Statistical analysis supervision; V.B. Visualisation; M.-D.M Visualisation and resources; H.-S.R. Statistical analysis; L.E. Funding acquisition and project administration. The published version of the manuscript has been read and approved by all authors.

Acknowledgments

The work was funded by the Romanian Ministry of Agriculture and Rural Development, grant number ADER 7.4.1, 2019-2022.

Conflicts of Interest

The authors declare that they do not have any conflict of interest.

REFERENCES

1. Bautista-Ortín AB, Jímenez-Martínez MD, Jurado R, Iniesta JA, Terrades S, Andrés A, Gómez-Plaza E. Application of high-power ultrasounds during red wine vinification. International Journal of Food Science and Technology, 2017, 52(6), 1314-1323.
2. Călugăr A, Babeş AC, Bunea CI, Pop TI, Tomoiagă L, Iliescu M. Oenological characterization of wines from grape clones created at Research Station for Viticulture and Enology Blaj, Romania. Agricultural Science. 2018; No 2(2018):50-6.
3. Chagas R, Monteiro S, Ferreira RB. Assessment of potential side effects of common fining agents used for white wine protein stabilizations. Am. J. Enol Vitic. ajev-2012; DOI: 10.5344/ajev.2012.12016
4. Chedea VS, Drăgulinescu AM, Tomoiagă LL, Bălăceanu C, Iliescu ML. Climate change and internet of things technologies-sustainable premises of extending the culture of the Amurg cultivar in Transylvania—a use case for Târnave vineyard. Sustainability. 2021; 13(15):8170.
5. Coldea TM, Mudura E, Chircu C, Borșa A. Chemical assessment of white wine during fermentation process, Bulletin UASVM Food Science and Technology. 2014; 71(1).
6. Comprehensive Reviews in Food Science and Food Safety. 2019; 18(2), 455–479. doi:10.1111/1541-4337.12422
7. Cudur F, Iliescu M, Comsa M, Popescu D, Cristea C. Soil type influence on yield quantity and quality at grape varieties for white wines obtained in the viticultural centre Blaj. Bulletin of University of Agricultural Science and Veterinary Medicine Cluj-Napoca – Horticulture. 2014; 71: 21-8.

8. Coroș MM, Pop AM & Popa AI. Vineyards and Wineries in Alba County, Romania towards Sustainable Business Development. Sustainability. 2019, 11, 4036.
9. Donici A, Bunea CI, Călugăr A, Harsan E & Bora FD. Investigation of the copper content in vineyard soil, grape, must and wine in the main vineyards of Romania: A preliminary study. Bull. UASVM Hortic, 76, 31–46, 2019.
10. EFSA. Scientific Opinion of EFSA prepared by the panel on food additives and nutrient sources added to food (ANS) on the use of natamycin (E 235) as a food additive. The EFSA Journal, 2009, 7, 1412–1437.
11. Elmaci SB, Gülgör G, Tokatli M, Erten H, Işci A, Özçelik F. Effectiveness of chitosan against wine-related microorganisms. Anton Leeuw. Int. J. G. 2015; 107, 675-686.
12. Er Y, Atasoy A. The Classification of white wine and red wine according to their physicochemical qualities. International Journal of Intelligent Systems and Applications in Engineering (IJISAE). 2016; 4 (Special Issue): 23-6.
13. Iliescu M, Tomoiaga L, Farago M, Comsa M. The nutrition of vine in Tarnave. Academic Press: Cluj-Napoca. 2010.
14. Lisanti MT, Blaiotta G, Nioi C, Moio L. Alternative methods to SO2 for microbiological stabilization of wine. Comprehensive Reviews in Food Science and Food Safety. 2019 Mar;18(2):455-79.
15. Marchante L, Marquez K, Contreras D, Izquierdo-Cañas PM, García-Romero E, Díaz-Maroto MC. Potential of Different Natural Antioxidant Substances to Inhibit the 1-Hydroxyethyl Radical in SO_2-Free Wines. Journal of Agricultural and Food Chemistry. 2020; doi:10.1021/acs.jafc.9b07024
16. Marchante L, Márquez K, Contreras D, Izquierdo-Cañas PM, García-Romero E, Díaz-Maroto MC. (2020). Impact of oenological antioxidant substances on the formation of 1-hydroxyethyl radical and phenolic composition in SO_2 free red wines. Journal of the Science of Food and Agriculture. 2020; doi:10.1002/jsfa.10374
17. Marchante L, Mena A, Izquierdo-Cañas PM, García-Romero E, Pérez-Coello MS, Díaz-Maroto MC. Effect of the prefermentative addition of chitosan in the nitrogenous fraction and secondary fermentation products of SO_2 free red wines. Journal of the Science of Food and Agriculture. 2020; DOI 10.1002/jsfa.10725
18. Nardi T, Vagnoli P, Minacci A, Gautier S, Sieczkowski N. Evaluating the impact of the fungal-origin chitosan preparation on *Brettanomyces bruxellemsis* in the context of wine aging. Wine Studies. 2014; 3:4574, 13-15.
19. Nunes C, Maricato É, Cunha Â, Rocha MAM, Santos S, Ferreira P, Silva MA, Rodrigues A, Amado O, Coimbra J, Silva D, Moreira A, Mendo S, Lopes da Sliva JA, Pereira E, Silva M, Rocha SM, Coimbra MD. Chitosan-genipin film, a sustainable methodology for wine preservation. Green Chemistry. 2016; 18, 5331-5341.
20. OIV-MA-AS323-04B. Compendium of international methods of analysis for spirituous beverage and alcohols – OIV. Sulfur dioxide (Iodometry)(Type-IV).
21. OIV-MA-AS313-01. Compendium of international methods of analysis for spirituous beverage and alcohols – OIV. Total Acidity (Type-I).
22. OIV-MA-AS313-02. Compendium of international methods of analysis for spirituous beverage and alcohols – OIV. Volatile Acidity (Type-I).
23. OIV-MA-AS2-03B, 2012. Compendium of international methods of analysis – OIV. Total Dry Matter - Type IV method.
24. OIV-MA-AS311-01A, 2009. Compendium of international methods of analysis– OIV. Reducing substances - Type IV method.
25. Pones P, Heras JM, Sieczkowski N, Canals JM, Zamora F. The oxygen consumption rate of an inactivate dry yeast (IDY) selected to protect wine from oxidation. Investigacion y Ciencia. Jan/Feb 2019.
26. Petrova B, Cartwright ZM, Edwards CG. Effectiveness of chitosan preparations against *Brettanomyces bruxellemsis* grown in culture media and red wines. OENO One. 2016; 50, 49-56.
27. Ribéreau-Gayon P, Glories Y, Maujean A, Dubourdieu D. Handbook of Enology: the chemistry of wine stabilization and treatments, 2nd edn., vol. 2, p. 152; 2006.
28. Răcoare HS, Iliescu ML, Tomoiagă LL, Comșa M, Sîrbu AD, Muntean MD, Chedea VS. The grapevine phenology and the climate changes in Tarnave vineyard. Horticulture. Vol. LXVI, No. 1, 2022.
29. Sîrbu A, Tomoiagă L, Chedea VS, Vasiu I, Iliescu M. New wines from Tarnave vineyard: Selena and Blasius. Bulletin of University of Agricultural Science and Veterinary Medicine Cluj-Napoca - Horticulture. 2022; 79(1): 70-73. DOI: 10.15835/buasvmcn-hort:2021.0044
30. Sîrbu AD, Tomoiagă LL, Răcoare HS, Şerbu FD, Chedea VS. Sulfur dioxide dinamic in Sauvignon blanc and Neuburger dry white wines. Bulletin of University of Agricultural Science and Veterinary Medicine Cluj-Napoca – Food Science and Technology. 2022; Vol. 79 No.2. DOI: 10.15835/buasvmcn-fst:2022-0012
31. Țârdea C. Chemistry and wine analysis. Ion Ionescu de la Brad Publishing House, Iaşi. 2007; 1213-1242.

32. Valera MJ, Sainz F, Mas A, Torija MJ. Effect of chitosan and SO_2 on viability of Acetobacter strains in wine. International Journal of Food Microbiology. 2017; 246, 1–4. doi:10.1016/j.ijfoodmicro.2017.01.022
33. Vally, H., Misso, N. L., & Madan V. Clinical effects of sulphite additives. Clinical and Experimental Allergy, 2009, 39, 1643–1651.
34. Ebulliometer. Quick wine alcohol determination by ebulliometry. Ref 160350D. Laboratoires Dujardin-Salleron. https://www.dujardin-salleron.com/documents/fiches/589b20d4a2332--160350---ft-ebulliometer-en.pdf
35. https://www.mapsland.com/europe/romania/large-map-of-romanian-wine-regions