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STUDY CONCERNING THE INFLUENCE OF SULPHUR DIOXIDE AND DIMETHYL DICARBONATE TREATMENTS IN WINE

Ioana CĂLIN[1], Camelia Elena LUCHIAN[1], Lucia Cintia COLIBABA[1], Elena-Cristina SCUTARAȘU[1], Andreea POPÎRDĂ[1], Vlăduț Ion CIMPOI[1], Cătălin Ioan ZAMFIR[2] , Valeriu V. COTEA[1]

[1]University of Agricultural Sciences and Veterinary Medicine "Ion Ionescu de la Brad" of Iaşi, 3 Mihail Sadoveanu Alley, Iași, Romania
[2]Research Centre for Oenology, 9 Mihail Sadoveanu Alley, Iaşi, Romania

Corresponding author email: catalin.zamfir@yahoo.com

Abstract

One of the current main challenges of modern enology is the effect of some preservative treatments on the final quality of wine regarding the chromatic and physical-chemical parameters. SO_2 is the most commonly used product in winemaking due to its antioxidant and antimicrobial effect. Nowadays, attempts are concentrated towards reducing sulphur dioxide and substituting it with other substances that play a significant role in wines stabilization.
For this study, twentyone variants were obtained from a blend of 'Muscat Ottonel' and 'Fetească regală' grape varieties at the experimental wine cellar of the Oenology Laboratory of the Faculty of Horticulture from Iași. The wines were treated with 6% SO_2 solution and dimethyl dicarbonate liquid solution, in various ratios.
This research aims to analyse the effect of stabilisation treatments with sulphur dioxide and dimethyl dicarbonate on physical-chemical and chromatic parameters of wines.
To carry out this experiment, Schizosaccharomyces spp. and Brettanomyces spp. were inoculated and the evolution of physical-chemical and chromatic parameters of wines was recorded.
The applied treatments have shown a synergic activity on quality, physical-chemical and chromatic parameters of wine.

Key words: *antiseptic, antimicrobial, colour parameters, conditioning treatments, quality wines.*

INTRODUCTION

In the last years, many studies have been focused on the impact of conditioning treatments on wine composition (Reynolds, 2010; Malfeito - Ferreira, 2010; Ough 1975). The conditioning treatments aim to eliminate or reduce the chemical, physical, microbiological and enzymatic degradation (Pomohaci *et. al*., 2001). A stabilized wine should not change its clarity after it has been bottled and sent out for consumption. In practice, stability is achieved by subjecting the wine to treatments and operations which, as a whole, form the conditioning process (Pomohaci *et al.*, 2001).
Modern researchers focus on the action of the oenological substances used for wines stabilization and their influence on physical-chemical composition and chromatic parameters. Many substances can be used to protect wine's composition and colour. This group includes those products which inactivate or eliminate microorganisms (Cotea, 1985). Sulphur dioxide is the most commonly used product in winemaking due to its antioxidant and antimicrobial effect, which is considered a necessity in winemaking (Țârdea, 2007). Wines can also be obtained without addition of sulphur dioxide, but in order to obtain a good quality product, they require special management and strict conservation conditions (Reynolds, 2010).
Nowadays, many studies focus on decreasing the total quantity of SO_2 in wines (Santos *et al*., 2011; Tambora *et al*., 2013). Producers are trying to decrease the added SO_2 by strictly managing its addition on grapes, must and wine, looking for new alternatives. Factors such as grapes health, chemical composition of musts, present microorganisms, temperature, and humidity must be monitored to prevent alteration of wines (Țârdea *et al*., 2000). Commission delegated regulation (EU) 2019/934 indicates the following maximum concentrations to be respected: red wines: 150 mg/L; white wines: 200 mg/L; sweet wines: up

to 350 mg/L total SO_2. In the last years, another substance used from modern winemakers was dimethyl dicarbonate (DMDC), known as yeast inhibitor and preservative for alcoholic beverages, especially for low alcohol wines.

DMDC is used as antimicrobial agent, its efficacy depending on the pH (lower pH requires less DMDC for equivalent antimicrobial action) (Ough *et al*., 1978). Wine pH plays a critical role in many aspects of winemaking, ie. microbiological stability of wines (Ribéreau-Gayon *et al.*, 1972).

The action of DMDC depends on numerous factors such as wine composition (ethanol, pH), temperature, yeast strains and initial inocculum (Bartowsky, 2009; Costa *et al*., 2008). After its addition in wines, it is immediately decomposed into alcohol and carbon dioxide. This compound has been proposed to be used instead of SO_2 in winemaking (Divol *et al*., 2005). DMDC was approved in the European Union for winemaking at a maximum of 200 mg/L at wines that contain more than 5 g/L of residual sugar (Regulation (EC) No 643/2006).

Nowadays, the researchers focus on a key parameter in winemaking: wine's colour (Basalekou *et al.*, 2017), one of the most important visual characteristics available since it provides a considerable amount of highly relevant information about its quality (Dobrei*,* 2017). Microorganisms can spoil wines' quality; therefore a continuous challenge to inhibit their growth is under study worldwide. It is now confirmed that *non-Saccharomyces* yeasts, considered in the past less important for the winemaking industry, can improve the composition and aroma profile in wines. Their contribution is represented by the ability to secrete enzymes and produce secondary metabolites, glycerol and ethanol, the release of mannoproteins. Moreover, they contribute to the colour stability of wine (Padilla *et al*., 2016).

In this study, a blend of 70% 'Muscat Ottonel' must and 30% 'Fetească regală' must from Iași vineyard was obtained. 'Muscat Ottonel' is known as an aromatic grape variety often used to obtain different blends due to its strong and fruity aromas. 'Fetească regală' is a semi-aromatic grape variety, appreciated for its fruity, wildflowers odor and high acidity. (Dobrei*,* 2017).

MATERIALS AND METHODS

Winemaking practices

'Muscat Ottonel' and 'Fetească regală' grapes were manually harvested in autumn of 2018 and processed in the Oenology Laboratory of the Faculty of Horticulture from Iași. Experimental samples were obtained by using the classic method for producing white wines. After the quantitative and qualitative reception, the grapes were crushed, destemmed and pressed using a hydraulic press. The resulted grape juice was collected in a stainless steel tank for the fermentation phase.

After this stage, white wine was divided into three aliquots in which different amounts of sulphur dioxide were administered: 40, 80, and 160 ppm. *Schizosaccharomyces pombe* spp. and *Brettanomyces* spp. were inoculated in various amounts (30 mg/L and 100 mg/L). *Schizosaccharomyces pombe* spp.yeast is able to metabolize malic acid causing an increase in alcohol concentration, favoring the formation of stable pigments in wine. It also has certain disadvantages such as low fermentation speed, the development of undesirable flavors and aromas (Loira *et al*., 2018). *Brettanomyces* spp. yeasts typically grow in wines with residual sugar content, appearing after completion of the alcoholic and malolactic fermentation, during aging of wine in barrels or bottles. The aroma characteristics of their spoilage-causing metabolites were described as burnt plastic, smoky, barnyard, horse sweat, leather and wet wool (Henick-Kling *et al*., 2000).

In this research, this type of yeasts was used to highlight their possible microbiological activity through the sensory, physical-chemical and chromatic changes of samples.

The resulted mixture was filtered (using sterile filters), bottled into 750 mL glass bottles and then different amounts of dimethyl dicarbonate (100 or 200 mg/750 mL) were added (Table 1). This substance plays a significant role in the wine stabilization process and prevents the growth of harmful microorganisms. Bottles were stored under controlled temperature conditions until physical-chemical analyses were performed.

For each aliquot, the control sample was represented by the wine mixture treated with different concentrations of SO_2 (40 ppm. - V0; 80 ppm. - V0′; 160 ppm. - V0″).

Standard chemical analyses were realized according to the International Organization of Vine and Wine methods (OIV, 2019).

Colour parameters were studied under the OIV standards and regulations. Evaluation of chromatic characteristics was made by the CIELab76 method using a Specord UV-Vis spectrophotometer.
This procedure measures different parameters, such as clarity, tonality, chromaticity, saturation, luminosity and hue (tint). The measurements were made at a wavelength of 300 and 800 nm (OIV-MA-AS2-11).
CIELab system characterizes colour variations as perceived by the human eye, representing a uniform 3-dimensional space defined by colorimetric coordinates L*, a* and b*.
L* represents the vertical axis, measured from 0 that means completely opaque to 100 (totally transparent). Parameters "+a*" red, "-a*" green, "+b*" yellow, "-b" blue were also registered (Rolle & Guidoni, 2007).

Statistical analyses were performed using Statgraphics Centurion XVI® software, (StatPoint Technologies, Inc, U.S.A.).

The alcoholic strength is one of the most important parameters defining wine's quality (Moreno & Peinado, 2012), which has an essential role in wine preservation (Jordão *et al*., 2015). Ethanol level influences the stability, taste and aroma profiles of wine. Its concentration depends on the accumulated sugar in grapes, climate changes and winemaking practices (Albertin *et. al*., 2017).
Wine's *total acidity* (TA) normally ranges from 5 to 7 g/L tartaric acid (Zoecklein *et al.* 1995).
Many factors can influence this parameter, such as grape variety, ripeness, wine-making technology, storage conditions and climatic conditions (Samoticha *et al.,* 2017).
pH has an essential influence on wine stabilization. Optimum pH levels in wines are between 3.2-3.6.
Volatile acidity refers to the total content of short-chain volatile acids removed from wine by steam distillation (Țârdea *et al*., 2000).

Table 1. Samples

Samples	Yeasts	Sulphur dioxide doses (ppm)	DMDC doses (mg/L)
V1	*Brettanomyces* spp.	40	0
V2			100
V3			200
V4	*Schizosaccharomyces pombe* spp.		0
V5			100
V6			200
V0	-		-
V7	*Brettanomyces* spp.	80	0
Vf8			100
V9			200
V10	-		-
V11	*Schizosaccharomyces pombe* spp.		100
V12			200
V0'	-		-
V13	*Brettanomyces* spp.	160	0
V14			100
V15			200
V16	*Schizosaccharomyces pombe* spp.		0
V17			100
V18			200
V0''	-		-

V0 - control sample with 40 ppm SO_2;
V0' - control sample with 80 ppm SO_2;
V0'' - control sample with 160 ppm SO_2.

Volatile acidity refers to the total content of short-chain volatile acids removed from wine by steam distillation (Țârdea *et al*., 2000). Acetic acid accounts for 95-99 % of the total volatile acidity and the rest is due to small quantities of lactic acid, propionic acid, butyric and formic acid.
Volatile acidity represents an important parameter in assessing the quality and health state of the final product (Gardner, 2015).
Malic and tartaric acids play a crucial role in winemaking, influencing organoleptic quality, physical-chemical parameters and microbial stability of wines (Volschenkla *et al*., 2006).
Malic acid is found in L-acid form in grapes, musts and wines. Its concentrations vary between 2-4 g/L, being influenced by grape varieties and climatic conditions.
During alcoholic fermentation, 10-15% of malic acid can be transformed by the

Saccharomyces spp. yeasts into ethyl alcohol and carbon dioxide.
Also, *Schizosaccharomyces species* metabolize malic acid up to 70-80%. Lactic bacteria can metabolize malic acid completely, resulting lactic acid and carbon dioxide (Redzepovic *et al.*, 2003).

RESULTS AND DISCUSSIONS

Physical-chemical assays

Physical-chemical parameters of samples were performed according to the International Organization of Vine and Wine Compendium methods of analysis (OIV, 2019). For each sample, the analyses was realised in triplicate for: ethanol content (% vol.), total acidity (g/L tartaric acid), volatile acidity (g/L acetic acid), pH (real acidity), density, total sugar (g/L), malic acid (g/L) and lactic acid (g/L).
The means and standard deviations of the physical-chemical parameters of wines are represented in Table 2.

Statistical analysis of physical-chemical and chromatic parameters of wines

In this study, ANOVA multifactor test was applied to construct a statistical model describing the impact of three categorical factors, such as X_j (sulphur dioxide, yeast type and dimethyl dicarbonate) on a dependent variable Y (physical-chemical parameters and CIE Lab parameters of wines). The results of the multifactor ANOVA statistical test on the physical-chemical parameters of wines were represented in Table 5. Dependent variables in this experiment are represented by the identified physical-chemical parameters while three factors influence their concentration (sulphur dioxide, yeast type and dimethyl dicarbonate content). In this sense, the contribution of each factor was statistically interpreted independent to the effects of all other factors.
The results showed that both treatments and inoculated yeasts have statistically significant influence (p-value < 0.05) on all physical-chemical parameters except for total acidity (p-value $= 0.1084$) and density (p-value $= 0.0984$).
The means and standard deviations of the chromatic parameters of wines are represented in Table 3. Wine colour measurements of each sample were performed in triplicate.
Dependent variables were represented by the identified chromatic parameters while three factors influence their values (sulphur dioxide, yeast type and dimethyl dicarbonate).
SO_2 content factor showed a statistical significant influence on the clarity and chromaticity “a” parameters (p-value < 0.05).
Synergic action of SO_2 content - yeast type (A-B) showed a statistical significant influence on all parameters except chromaticity “b” and chroma (saturation).

CONCLUSIONS

The statistical results suggest a synergic action of administrated treatments (sulphur dioxide and dimethyl dicarbonate) having important effects on chromatic and physical-chemical parameters. Regarding the physical-chemical parameters, only total acidity and density are not statistically significant influenced.
The inoculated microorganisms also played an important role in the development of the final colour and stability of wines.
Since this article aimed to evaluate the influence of sulphur dioxide and DMDC on the evolution of chromatic and chemical parameters in white wines, the results confirm that the treatments have a significant effect on wine’s quality.

Table 2. The means and standard deviations of the physical-chemical parameters of samples

Samples	Ethanol % vol. alc.	Total acidity (g tartaric acid/L)	Volatile acidity (g acetic acid/L)	Total sugar g/L	Density	pH	Malic acid g/L	Lactic acid g/L
V0	14.20±0.17	4.90±0.16	0.39±0.01	20.70±0.01	0.9968±0.0001	3.42±0.02	0.10±0.03	1.10±0.01
V1	14.80±0.15	4.90±0.13	0.23±0.02	22.00±0.02	0.9966±0.0002	3.39±0.02	0.20±0.02	0.90±0.03
V2	14.70±0.19	5.66±0.12	0.21±0.01	21.00±0.02	0.9966±0.0001	3.30±0.03	1.70±0.02	0.00±0
V3	14.90±0.15	5.97±0.15	0.18±0.03	20.50±0.01	0.9970±0.0002	3.23±0.02	2.30±0.03	0.00±0
V4	15.00±0.17	4.90±0.16	0.21±0.02	21.70±0.02	0.9967±0.0003	3.38±0.02	0.40±0.03	0.60±0.04
V5	14.90±0.19	6.12±0.18	0.20±0.02	21.20±0.01	0.9972±0.0003	3.25±0.01	2.50±0.02	0.00±0
V6	14.80±0.18	6.43±0.17	0.16±0.01	19.20±0.02	0.9961±0.0002	3.26±0.02	2.90±0.03	0.00±0
V0’	14.90±0.17	6.28±0.15	0.17±0.03	20.70±0.04	0.9969±0.0004	3.25±0.03	2.60±0.02	0.00±0
V7	14.90±0.18	6.12±0.14	0.18±0.02	21.00±0.01	0.9971±0.0003	3.21±0.02	2.40±0.02	0.00±0
V8	14.80±0.16	6.43±0.15	0.16±0.04	19.60±0.02	0.9969±0.0002	3.27±0.02	2.80±0.02	0.00±0
V9	15.10±0.14	6.12±0.17	0.20±0.02	21.40±0.02	0.9967±0.0001	3.21±0.01	2.40±0.03	0.00±0
V10	14.90±0.15	6.43±0.16	0.13±0.01	20.10±0.01	0.9971±0.0001	3.27±0.02	2.90±0.03	0.00±0
V11	15.00±0.17	6.12±0.18	0.17±0.02	20.80±0.01	0.9968±0.0002	3.22±0.03	2.40±0.02	0.00±0
V12	14.90±0.16	6.12±0.19	0.13±0.03	19.70±0.04	0.9965±0.0003	3.22±0.01	2.50±0.02	0.00±0
V0’’	15.20±0.18	6.12±0.17	0.17±0.04	17.50±0.03	0.9954±0.0004	3.19±0.01	2.40±0.03	0.00±0
V13	15.20±0.19	6.28±0.19	0.16±0.02	19.50±0.01	0.9959±0.0002	3.18±0.02	2.40±0.02	0.00±0
V14	15.20±0.16	6.28±0.20	0.16±0.01	19.20±0.02	0.9958±0.0003	3.18±0.03	2.50±0.02	0.00±0
V15	15.00±0.17	6.43±0.18	0.13±0.03	18.40±0.04	0.9954±0.0003	3.22±0.02	2.70±0.02	0.00±0
V16	15.00±0.18	6.28±0.17	0.14±0.02	20.60±0.03	0.9966±0.0002	3.20±0.01	2.60±0.02	0.00±0
V17	15.10±0.16	6.12±0.19	0.17±0.01	20.50±0.02	0.9966±0.0003	3.17±0.03	2.40±0.02	0.00±0
V18	15.10±0.17	6.12±0.20	0.17±0.02	20.80±0.03	0.9967±0.0002	3.17±0.03	2.50±0.02	0.00±0

Table 3. The means and standard deviations of the chromatic parameters of samples

Sample	Clarity L*	Chromaticity		Chroma C	Tonality H	Luminosity	Tint	ΔE	ΔH
		a*	b*						
V0	55.33±0.24	8.86±0.18	31.29±0.12	32.52±0.14	74.18±0.22	2.44±0.05	1.68±0.03	64.18±0.20	8.86±0.24
V1	63.47±0.22	8.44±0.19	32.4±0.11	33.47±0.12	75.04±0.20	1.97±0.05	1.78±0.04	71.76±0.18	8.44±023
V2	60.89±0.20	8.74±0.17	32.46±0.13	33.62±0.11	74.93±0.19	2.12±0.04	1.75±0.02	69.55±0.20	8.74±0.23
V3	64.18±0.22	8.38±0.18	31.98±0.12	33.06±0.14	75.32±0.20	1.92±0.02	1.77±0.05	72.19±0.19	8.38±0.22
V4	59.76±0.20	9.00±0.16	32.82±0.12	34.04±0.15	74.68±0.21	2.19±0.03	1.74±0.03	68.77±018	9.00±0.23
V5	67.38±0.19	7.95±0.16	32.15±0.12	33.12±0.15	76.1±0.18	1.75±0.03	1.83±0.02	75.08±0.20	7.95±0.22
V6	60.21±0.17	9.33±0.18	30.65±0.13	32.04±0.13	73.06±0.15	2.12±0.05	1.68±0.04	68.20±0.19	9.33±0.23
V0’	96.62±0.18	1.17±0.19	7.39±0.14	7.49±0.15	80.97±0.19	0.19±0.02	2.4±0.06	96.91±0.22	1.17±0.20
V7	96.54±0.19	1.29±0.17	7.48±0.14	7.59±0.14	80.18±0.18	0.19±0.02	2.37±0.07	96.84±0.21	1.29±0.21
V8	96.66±0.17	1.09±0.17	7.37±0.13	7.45±0.13	81.63±0.20	0.19±0.03	2.42±0.03	96.95±0.19	1.09±0.20
V9	96.66±0.16	1.13±0.16	7.29±0.11	7.37±0.13	81.17±0.16	0.19±0.05	2.4±0.05	96.94±0.19	1.13±0.19
V10	96.86±0.18	0.96±0.17	7.17±0.12	7.23±0.12	82.35±0.17	0.18±0.09	2.49±0.03	97.13±0.20	0.96±0.21
V11	96.66±0.19	1.15±0.18	7.41±0.11	7.5±0.14	81.19±0.18	0.19±0.07	2.41±0.04	96.95±0.19	1.15±0.19
V12	96.66±0.17	1.15±0.17	7.4±0.11	7.49±0.13	81.18±0.17	0.19±0.08	2.41±0.05	96.95±0.18	1.15±0.19
V0’’	98.15±0.16	-0.25±0.17	5.67±0.1	5.7±0.14	-87.5±0.20	0.12±0.05	3.48±0.02	98.31±0.20	0.25±0.20
V13	98.21±0.15	-0.36±0.16	5.79±0.09	5.8±0.13	-86.39±0.16	0.13±0.07	3.66±0.02	98.38±0.17	0.36±0.21
V14	98.22±0.13	-0.34±0.15	5.8±0.1	5.81±0.12	-86.62±0.18	0.13±0.07	3.63±0.04	98.39±0.18	0.34±0.21
V15	98.09±0.14	-0.28±0.15	5.85±0.11	5.86±0.13	-87.27±0.19	0.13±0.05	3.46±0.05	98.26±0.18	0.28±0.19
V16	98.35±0.15	-0.31±0.14	5.54±0.09	5.55±0.12	-86.81±0.20	0.12±0.04	3.69±0.05	98.51±0.17	0.31±0.20
V17	98.2±0.16	-0.29±0.13	5.61±0.08	5.62±0.13	-87.09±0.19	0.12±0.05	3.52±0.04	98.36±0.19	0.29±0.19
V18	98.3±0.16	-0.31±0.12	5.51±0.08	5.52±0.11	-86.79±0.20	0.12±0.04	3.63±0.03	98.45±0.19	0.31±0.18

“ΔE” represents colorimetric difference; “ΔH” represents tonality difference.

Table 4. Results of the multifactor ANOVA statistical test on the physical-chemical parameters of wines

FACTORS	ETHANOL CONTENT		TOTAL ACIDITY		VOLATILE ACIDITY		TOTAL SUGAR		DENSITY		pH		MALIC ACID		LACTIC ACID	
	F- ratio	*P*-value	F- ratio	*P*-value	F- ratio	*P*-value	F- ratio	*P*-value	F- ratio	*P*-value	F- ratio	*P*-value	F- ratio	*P*-value	F- ratio	*P*-value
A- SO_2 CONTENT	34.85*	0.0000	26.22*	0.0000	74.50*	0.0000	34.40*	0.0000	39.18*	0.0000	49.83*	0.0000	29.79*	0.0000	35.37*	0.0000
B-YEAST TYPE	5.01*	0.0101	2.32^{ns}	0.1084	29.93*	0.0000	6.02*	0.0044	5.45*	0.0070	4.24*	0.0194	4.01*	0.0237	7.30*	0.0016
C-DMDC CONTENT	3.24*	0.0294	15.21*	0.0000	19.1*	0.0000	7.64*	0.0000	2.21^{ns}	0.0984	19.31*	0.0000	55.72*	0.0000	166.03*	0.0000
A-B	7.57*	0.0001	2.64*	0.0438	20.85*	0.0000	13.08*	0.0000	9.75*	0.0000	2.70*	0.0402	3.48*	0.0134	7.30*	0.0001
A-C	5.06*	0.0004	19.04*	0.0000	13.96*	0.0000	6.21*	0.0001	6.21*	0.0001	13.61*	0.0000	60.09*	0.0000	166.03*	0.0000

Table 5. Results of the multifactor ANOVA statistical test on the chromatic parameters of wines

FACTORS	CLARITY		CHROMATICITY (±a)		CHROMATICITY (±b)		CHROMA	
	F- ratio	*P*-value	F- ratio	*P*-value	F- ratio	*P*-value	F- ratio	*P*-value
A- SO_2 CONTENT	3130.89*	0.0000	4418.17*	0.0000	21906.18*	0.0000	27292.76*	0.0000
B-YEAST TYPE	9.95*	0.0002	0.82^{ns}	0.4477	4.04*	0.0233	3.51*	0.0368
C-DMDC CONTENT	8.44*	0.0001	1.68^{ns}	0.1830	9.71*	0.0000	8.53*	0.0001
A-B	9.50*	0.0000	0.85^{ns}	0.4987	2.18^{ns}	0.0835	1.96^{ns}	0.1138
A-C	8.15*	0.0000	1.21^{ns}	0.3179	9.94*	0.0000	9.21*	0.0000

The superscript symbol * indicates that these factors with *p*-value less than 0.05 have a statistically significant effect on the parameter at the 95.0 % confidence level.
Data are means of triplicate determination ± standard deviation over the three replications in wine sample. The superscript letters *n.s.* indicates that the factor does not have a statistically significant influence. All F-ratios are based on the residual mean square error.

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***Commission regulation (EC) No. 643/2006 of 27 April 2006 amending Regulation (EC) No. 1622/2000 laying down certain detailed rules for implementing Regulation (EC) No. 1493/1999 on the common organisation of the market in wine and establishing a Community code of oenological practices and processes, and Regulation (EC) No. 884/2001 laying down detailed rules of application

concerning the documents accompanying the carriage of wine products and the records to be kept in the wine sector.

***Commission delegated regulation (EU) 2019/934 of 12 March 2019 supplementing Regulation (EU) No 1308/2013 of the European Parliament and of the Council as regards wine-growing areas where the alcoholic strength may be increased, authorised oenological practices and restrictions applicable to the production and conservation of grapevine products, the minimum percentage of alcohol for by-products and their disposal, and publication of OIV files.

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