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Impact of technological operations on oxygen consumption during wine production

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ABSTRACT

Oxygen plays a crucial role in all stages of wine production. The aim of this study was to quantify dissolved oxygen in filtered wines trained on fine lees during different technological operations such as racking, coarse filtration, stabilisation of thermolabile proteins, and sterile filtration and bottling. The most significant oxygenation of wine occurs during filtration (1.9–3.57 mg L^{-1}) and during bottling (2.99–4.12 mg L^{-1}). At the same time, oxygen affects the phenolic composition, antioxidant activity and sulphur dioxide.

Understanding and being able to use oxygen correctly during wine production can lead to a reduction in the doses of sulphur dioxide used. It has been shown that wines trained on fine lees are more able to withstand oxygen and, therefore, the sulphur dioxide doses can be reduced substantially. The experiment, in which two different winemaking technologies were observed, was carried out on the Welschriesling variety using both stainless steel tanks and oak barrels.

KEYWORDS

oxygen, wine oxidation, yeast lees, phenolic compounds, sulphur dioxide

1. INTRODUCTION

The winemaking process involves many stages in which the quantity of oxygen varies. The least amount of oxygen is present in the wine during fermentation, when the wine is protected from

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oxygen, since it is quickly consumed by the yeast or displaced by carbon dioxide (Valade et al., 2006). Another stage with low oxygen content in wine is the sedimentation of the lees, when almost all the oxygen is consumed by the micro-organisms. After these stages, white wine is very susceptible to oxidation, and it is the technological processes following fermentation that will be the subject of our research.

Under anaerobic conditions, redox systems are in equilibrium at certain time intervals. Any supply of oxygen to the wine immediately disturbs the equilibrium state. Oxygen diffuses into musts or wines and reacts with easily oxidisable compounds and oxidises them. In these reactions, peroxides can be formed that affect other oxidation and radical processes in the wine (Ailer et al., 2022). Oxygen enters the wine both actively and passively, with the active input being processes such as racking, bâttonage, clarification, and filtration of the wine, and the passive input being the diffusion of oxygen through holes, seals, and stoppers during storage of the wine whether in process vessels or in bottles. The subsequent positive or negative effect on the wine depends on the amount of dissolved oxygen, the timing of oxygenation, and physical conditions such as pressure and temperature. Temperature influences the level of saturation of dissolved oxygen, with higher concentrations dissolving at lower temperatures, as low as around 5 °C (Du Toit et al., 2006; Valade et al., 2006). Castellari et al. (2004) stated that oenological operations can be divided into low and high oxygen enrichment operations in terms of the amount of oxygen supplied to the wine. In these processes, wine can be exposed to doses ranging from 0.1 mg L^{-1}–7.5 mg L^{-1} (Vivas et al., 2003).

The oxygen supplied also influences the chemical composition of the wine; the different components of the wine have varying abilities to react with oxygen and, therefore, their quantity in the wine changes due to oxidation. The substances that react with oxygen are mainly phenolic substances, glutathione, ascorbic acid, ethanol, and fatty acids. Technology of targeted oxygenation of must or mash as a way of decreasing polyphenols in white wines without impact on sensorial changes was published by Ailer et al. (2021). The oxygen dissolved in the wine is in a non-reactive triplet state that has minimal potential to react directly with most of these compounds. The reactivity is usually conditioned by the presence of an oxidation catalyst, which in wine is iron and copper ions. The reaction between dissolved oxygen and the metal ions produces the superoxidised anion-radical $O_2^{\bullet-}$. However, this radical is not highly reactive at the pH of the wine and, therefore, reacts mainly with phenolic substances to form o-quinones and hydrogen peroxide. These products then take part in other chemical reactions in the wine (Danilewicz, 2013; Carrascon et al., 2017; Walls, 2020).

Previous studies have described the effect of oxygen on wine during bottling and storage, the permeability of different types of closures and the subsequent effect of oxygen on wine, or even the effect of adding a small amount of oxygen to wine during fermentation (Karbowiak et al., 2010; Prusova and Baron, 2018; Tarko, 2020).

The aim of this study was to compare the amount of dissolved oxygen in wines produced by conventional technology and in maturation of wines on yeast lees during the different technological stages such as sedimentation, racking, and filtration, in a stainless tank and in barrique barrels. The novelty of the study is that it determines which operations supply a critical amount of oxygen to the wine, the effect of yeast lees due to their ability to absorb oxygen, and the effect of the oxygen on the antioxidant activity of the wine.

2. MATERIALS AND METHODS

2.1. Design of experiment

The experiment was carried out in real conditions of the winery on the variety Welschriesling in a total volume of 1650 L in two variants: technology with the use of fermentation lees and technology with immediate filtration after the end of fermentation. Each of the variants was divided into two types of container (600 L stainless steel container and 225 L barrique) and repeated in two successive vintages (see Table 1).

2.1.1. Technology using fine lees. After pressing, the must was settled (24 h at 7 °C) and divided into selected containers (600 L stainless steel containers and 225 L barriques). The must was inoculated with the commercial active dry yeast Oenoferm Klosterneuburg (Erbslöh Geisenheim AG, Geisenheim, Germany) at a dose of 15 g hL^{-1}, and fermentation was carried out at a temperature of 18–20 °C. After completion of fermentation (fermentation lasted 14 days), the first measurement of dissolved oxygen (DO) was carried out with a NomaSense immersion probe. At the same time as the measurements, samples were taken for laboratory analysis, and the wine was racked from the coarse lees and the DO value was determined again. During production, the wine was stirred on medium lees and the DO concentration and the amount of free and total sulphur dioxide (SO_2) were monitored. After 6 months, the wine was decanted from the lees, clarified with a dose of 80 g hL^{-1} of Bentostab (IOC, Épernay, France), and treated with SO_2 (Supersolfosol - Antioxidant 40%, Esseco S.r.l., Trecate, Italy; 15 mL hL^{-1}), followed by sterile filtration using a plate filter (Becopad 170, Eaton, Dublin, Ireland), stabilisation of the free SO_2 level (Supersolfosol - Antioxidant 40%, Esseco S.r.l., Trecate, Italy; 5 mL hL^{-1}), and bottling. Bottling was performed using a bottling line, without the use of inert gas.

2.1.2. Technology with filtration after fermentation. After pressing, the must was settled and separated into 600 L stainless steel container and 225 L barrique. Inoculation was carried out using the neutral yeast strain Oenoferm Klosterneuburg (Erbslöh Geisenheim AG, Geisenheim, Germany) at a controlled temperature of 18–20 °C. The first measurement of DO by dip probe was carried out after the end of fermentation. At the same time, samples were taken, and the wine was filtered using a plate filter (Becopad 450, Eaton, Dublin, Ireland) and (Supersolfosol - Antioxidant 40%, Esseco S.r.l., Trecate, Italy; 10 mL hL^{-1}). This was followed by clarification with a dose of 80 g L^{-1} of Bentostab (IOC, Épernay, France) and treatment with SO_2 (Supersolfosol - Antioxidant 40%, Esseco S.r.l., Trecate, Italy; 10 mL hL^{-1}). The wine was then sterile filtered (Becopad 170, Eaton, Dublin, Ireland), the SO_2 level stabilised (Supersolfosol - Antioxidant 40%, Esseco S.r.l., Trecate, Italy; 10 mL hL^{-1}), and bottled. Bottling took place using a bottling line, without the use of inert gas. At each handling of the wine, the amount of DO was measured.

Table 1. Design of experiment

Welschriesling			
Stainless steel tank 600 L		Barrique 225 L	
Filtered (8 μm)	With fine lees	Filtered (8 μm)	With fine lees

2.2. NomaSense oxygen measurement

The NomaSense portable analyser (Nomacorc, Florence, Italy) was used to measure oxygen using luminescence technology combined with remote sensors and other accessories to measure the oxygen level in wine (P300andP6000, 2018; Wine Business, 2019).

2.3. Determination of SO_2

The procedure OIV-MA-AS323-O4B: R 2009 was used for determination of the free and bound forms of SO_2.

2.4. Determination of total phenols

The total phenols were determined using the modified Folin–Ciocalteu method. Samples of 12 and 10 μL Folin–Ciocalteu reagent were added to 198 μL of water. After 36 s, 30 μL of 20% sodium carbonate solution was added. The absorbance at 700 nm was measured after 600 s. The concentration of total phenols was calculated on a calibration curve using gallic acid as the standard (25–1,000 mg L^{-1}). The results were expressed in the form of gallic acid equivalents (GAE) (Waterman and Mole, 1994; Sochor et al., 2014; Sochorova et al., 2020).

2.5. Determination of antioxidant activity by FRAP method

A 12 μL sample was added to 198 μL of alkalic buffer containing 200 mM sodium acetate and treated with acetic acid to a pH of 3.6, 20 μL of 20 mM $FeCl_3$ and 20 μL 10 mM 2,4,6-tripyridyl-s-triazine solution in 40 mM HCl. The reduction force was calculated from the calibration curve using ascorbic acid (0.1–3 mM) or gallic acid (10–300 mg L^{-1}) as standard. Results are expressed in GAE (Pulido et al., 2000).

2.6. Statistical evaluation

After the measurements, the results were processed by Microsoft Excel (Microsoft 365®) and statistically evaluated using the software Statistica 12 (StatSoft CR s.r.o.). ANOVA and factorial ANOVA methods are commonly used for the comparison of the classes of samples based on different features (in our case, the values of the sensory attributes). The ANOVA method with the use of post hoc tests – computed after the primer analysis – makes a pairwise comparison of the average values of the different groups of samples.

3. RESULTS AND DISCUSSION

The amount of oxygen in wine is constantly changing depending on the movement of the wine, the addition of oenological preparations, and the chemical composition of the wine itself. During the experiment, dissolved oxygen was always measured before and immediately after the technological operation. Additionally, after the technological operation, samples were taken for chemical analysis, since the kinetics of oxygen dissolution are very high and oxygen reacts with the various chemical components of the wine. During the study, it was found that wines mixed on yeast lees contained lower amounts of dissolved oxygen or processed the supplied oxygen more quickly (see Table 2). Furthermore, it was found that wines trained in barrique

Table 2. The average content of dissolved oxygen (mg L^{-1}) before and after each oenological treatment in the 2020 and 2021 vintages

		DO (mg L^{-1}) 2020			DO (mg L^{-1}) 2021			
	Treatment	Before treatment	After treatment	Enrichment in (mg L^{-1})	Before treatment	After treatment	Enrichment in (mg L^{-1})	Post hoc $\alpha = 0.05$
Stainless steel tank FILTR.	Rac	0.17 ± 0.12	0.456 ± 0.25	0.29	0.23 ± 0.15	0.48 ± 0.18	0.26	0.626
	Filt	0.01 ± 0.0	5.886 ± 0.13	5.87	0.02 ± 0.01	5.78 ± 0.03	5.76	0.485
	ProtSt	0.07 ± 0.01	0.313 ± 0.02	0.3	0.01 ± 0.01	0.37 ± 0.11	0.36	0.066
	StFiltr	0.04 ± 0.02	2.137 ± 0.06	2.10	0.05 ± 0.04	2.24 ± 0.23	2.19	0.395
	Bot	0.01 ± 0.0	4.07 ± 0.01	4.06	0.01 ± 0.00	4.03 ± 0.04	4.02	0.718
Stainless steel tank FINE LEES	Rac	0.07 ± 0.1	0.03 ± 0.02	0	0.05 ± 0.06	0.03 ± 0.01	0.01	0.981
	Bât	0.01 ± 0.0	0.03 ± 0.02	0.02	0.01 ± 0.01	0.02 ± 0.01	0.02	0.961
	ProtSt	0.12 ± 0.2	0.03 ± 0.03	0	0.09 ± 0.14	0.03 ± 0.02	0.02	0.818
	StFiltr	0.01 ± 0.0	1.78 ± 0.02	1.76	0.01 ± 0.00	1.61 ± 0.17	1.6	0.097
	Bot	0.58 ± 0.0	3.24 ± 0.01	3.8	0.01 ± 0.00	3.18 ± 0.04	3.17	0.000
Barrique FILTR	Rac	0.04 ± 0.05	0.337 ± 0.08	0.29	0.04 ± 0.05	0.48 ± 0.15	0.47	0.221
	Filt	0.01 ± 0.0	4.133 ± 0.18	4.12	0.01 ± 0.00	4.38 ± 0.43	4.36	0.528
	ProtSt	0.13 ± 0.04	0.38 ± 0.02	0.25	0.13 ± 0.05	0.39 ± 0.02	0.26	0.948
	StFiltr	0.01 ± 0.0	3.58 ± 0.04	3.57	0.01 ± 0.01	3.55 ± 0.09	3.53	0.694
	Bot	0.01 ± 0.01	4.13 ± 0.14	4.12	0.01 ± 0.00	4.08 ± 0.02	4.07	0.646
Barrique FINE LEES	Rac	0.01 ± 0.0	0.04 ± 0.02	0	0.01 ± 0.00	0.04 ± 0.01	0.03	0.981
	Bât	0.00 ± 0.0	0.01 ± 0.01	0.01	0.07 ± 0.10	0.01 ± 0.01	0.01	0.974
	ProtSt	0.98 ± 0.12	0.01 ± 0.08	0.9	0.01 ± 0.01	0.98 ± 0.08	0.91	0.922
	StFiltr	0.01 ± 0.0	1.94 ± 0.03	1.92	0.01 ± 0.00	1.74 ± 0.18	1.73	0.055
	Bot	0.01 ± 0.01	3.00 ± 0.02	2.99	0.01 ± 0.00	3.01 ± 0.04	3.0	0.870

Note: Results are expressed as the mean value of three measurements ± standard deviation. Abbreviations of oenological treatments: Rac: racking; Filt: filtration; Bât: bâtonnage; ProtSt: protein stabilisation; StFiltr: sterile filtration; Bot: bottling.

barrels dealt with dissolved oxygen more quickly than those in stainless steel tanks. This confirms the results of research conducted by Fornairon-Bonnefond et al. (2003), which indicated that fermentation lees show high oxygen consumption during autolysis. Table 2 presents a comparison of two consecutive vintages. A post hoc analysis was performed for this repetition.

In Figs 1–4, it can be seen that the highest degree of oxygenation occurs in the filtered variant. Compared to the unfiltered variant, the same vessel can hold up to 1 mg L^{-1} more dissolved oxygen during operations such as filtration and bottling. Similar results can be observed for the barrique variants. It is clear from this observation that wines aged on yeast lees are better able to withstand oxygen during the various technological operations. This is confirmed by the previously mentioned research conducted by Fornairon-Bonnefond and Salmon (2003). Measurements of the amount of dissolved oxygen during the technological operations of wine production have been carried out by many researchers, but never when comparing different technologies. Interesting results were obtained by Catarino et al. (2014) when comparing the oxygenation of white and red wine and to findings of Vidal et al. (2001), who compared filtration types. They divided the filtration process using silica filtration into three critical parts, in which they measured the amount of dissolved oxygen. They found that the highest oxygenation occurred in the first stage of filtration (approximately the first 15 min). At that time, the wine was enriched by about 2–4 mg L^{-1} of oxygen. In the middle part of the filtration process, the dissolved oxygen content was between 0.1 and 0.7 mg L^{-1}. The critical point was the emptying of the filter, when the wine might be enriched by up to 4.9 mg L^{-1} of oxygen. After filtration, the wine might reach a level of up to 1.7 mg L^{-1} dissolved oxygen. Du Toit et al. (2006) reported values for filtration of 4–7 mg L^{-1} of oxygen, thus confirming

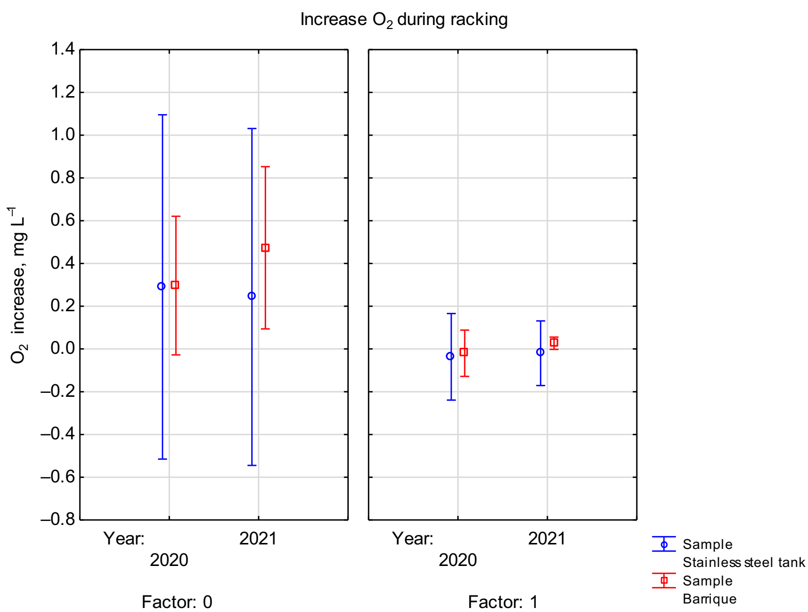

Fig. 1. Oxygenation rate of variants in racking. Factor 0 (filtered variant), factor 1 (fine lees)

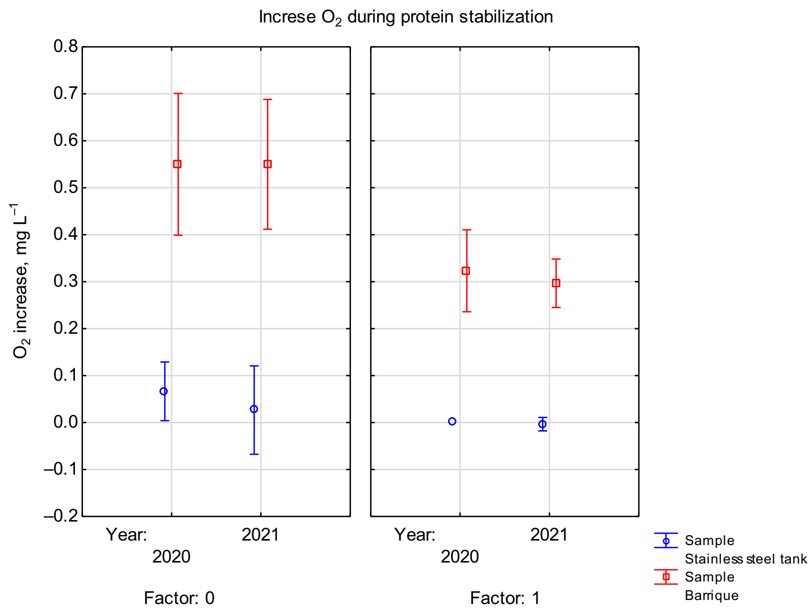

Fig. 2. Oxygenation rate of variants in protein stabilisation. Factor 0 (filtered variant), factor 1 (fine lees)

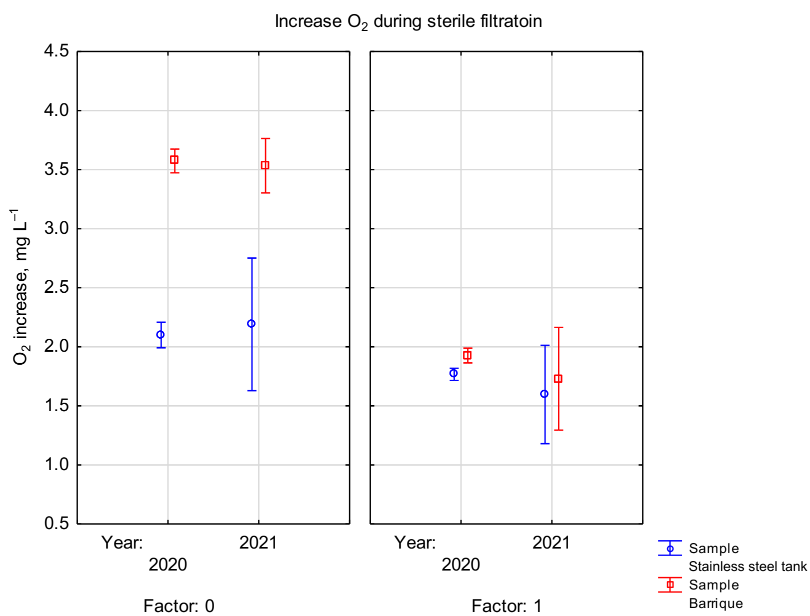

Fig. 3. Oxygenation rate of variants in sterile filtration. Factor 0 (filtered variant), factor 1 (fine lees)

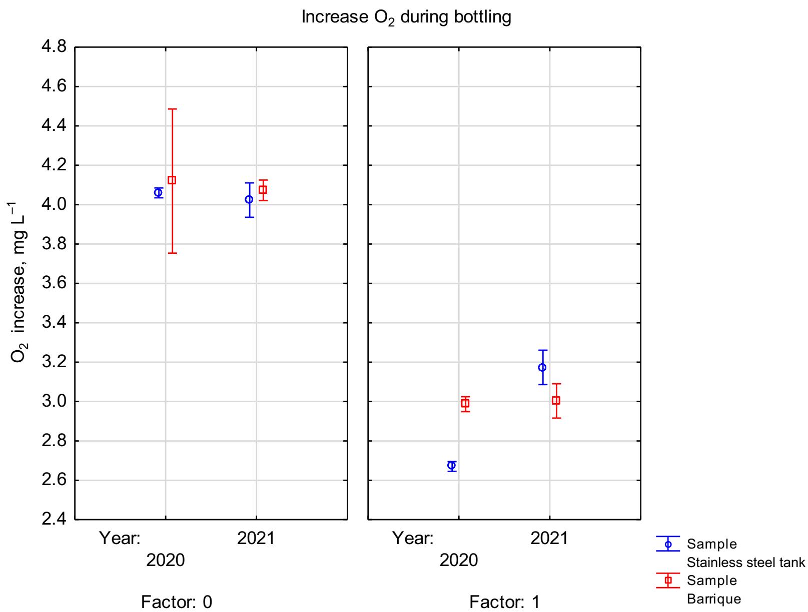

Fig. 4. Oxygenation rate of variants during bottling. Factor 0 (filtered variant), factor 1 (fine lees)

our conclusion that filtration is one of the most critical operations in wine production. Our measurements were then carried out using plate filtration, which has similar critical points to the above-mentioned silica filtration. During our measurements, values ranging from 1.7 to 4.1 mg L^{-1} were obtained, the higher value being measured immediately after the start of filtration. The total amount of dissolved oxygen in the wine after the end of filtration ranged from 1.7 to 2.02 mg L^{-1} depending on the variant observed (see Table 2).

Simultaneously with the oxygen measurements, the concentrations of free and total sulphur dioxide were determined. For the variants on fermentation lees, all variants showed significantly lower total sulphur dioxide content than for the filtered variants (Table 3). Conversely, for free sulphur dioxide, the aim was to keep the level approximately the same; however, a large decrease in free sulphur dioxide content for the filtered variants in the stainless steel tank could be seen. This is related to the low ability of the wine to withstand exposure to oxygen, as mentioned earlier. The considerably lower total sulphur dioxide content is also supported by the findings of Schneider et al. (2016), whose research into oxygen consumption by fermentation lees suggested that traditional bâtonnage stimulates a reduction in the sulphur dioxide used. This finding is confirmed by Valade et al. (2006), who stated that fermentation lees has a significant reducing power that protects wine from oxidation.

The correlation between chemical and sensory data is not a used concept in food research. Numerous food and beverage flavour research groups, chemical analysis specialists, and sensory analysis experts have investigated the various factors linking the concentration of ingredients with sensory responses (Ailer et al., 2020). Phenolic compounds are directly related to wine quality parameters. They not only contribute to the organoleptic characteristics of wine, but they

Table 3. Amount of free and total sulphur dioxide for all variants

	Treatment	2020 Free $SO_2 \pm$ SD (mg L^{-1})	2020 Total $SO_2 \pm$ SD (mg L^{-1})	2021 Free SO_2) $\pm$ SD (mg L^{-1})	2021 Total $SO_2 \pm$ SD (mg L^{-1})
Stainless steel tank FILTERED	Rac	7.7 ± 0.58	80.3 ± 0.58	9.7 ± 0.58	59.7 ± 0.58
	Filt	5.0 ± 0.00	80.3 ± 0.58	9.0 ± 1.00	80.3 ± 0.58
	ProtSt	15.0 ± 0.00	100.0 ± 0.00	20.7 ± 0.58	95.3 ± 0.58
	StFilt	9.3 ± 0.58	101.3 ± 1.15	20.3 ± 0.58	100.3 ± 0.58
	Bot	25.0 ± 0.00	130.3 ± 0.58	18.3 ± 0.58	100.3 ± 0.58
Stainless steel tank FINE LEES	Rac	11.3 ± 0.58	76.0 ± 0.00	10.0 ± 0.00	50.3 ± 0.58
	ProtSt	16.0 ± 0.00	91.3 ± 0.58	25.0 ± 0.00	64.7 ± 0.58
	StFilt	16.0 ± 0.00	91.7 ± 0.58	18.0 ± 0.00	65.0 ± 0.00
	Bot	22.0 ± 0.00	97.7 ± 0.58	20.0 ± 0.00	69.7 ± 0.58
Barrique FILTERED	Rac	3.7 ± 0.58	62.0 ± 0.00	4.0 ± 0.00	58.7 ± 0.58
	Filt	4.7 ± 0.58	62.7 ± 0.58	14.7 ± 0.58	65.0 ± 0.00
	ProtSt	2.7 ± 0.58	64.7 ± 0.58	20.0 ± 0.00	80.7 ± 1.15
	StFilt	20.3 ± 0.58	84.7 ± 0.58	19.7 ± 0.58	89.7 ± 0.58
	Bot	24.0 ± 0.00	90.7 ± 1.15	18.3 ± 0.58	90.0 ± 0.00
Barrique FINE LEES	Rac	3.7 ± 0.58	60.3 ± 0.58	4.7 ± 0.58	49.7 ± 0.58
	ProtSt	2.7 ± 0.58	61.3 ± 0.58	2.7 ± 0.58	49.7 ± 0.58
	StFilt	20.3 ± 0.58	80.0 ± 0.00	20.0 ± 0.00	70.0 ± 0.00
	Bot	22.0 ± 0.00	84.7 ± 0.58	18.3 ± 0.58	69.3 ± 1.15

Note: Results are expressed as the mean value of three measurements ± standard deviation. Abbreviations od enological treatments: Rac: racking; Filt: filtration; Bât: bâtonnage; ProtSt: protein stabilization; StFiltr: sterile filtration; Bot: bottling.

are also the main cause of colour changes in wine due to oxidation (Pérez-Serradilla and De Castro, 2008). If we oxidise mash or must by its exposure to atmospheric oxygen for a while without the use of sulphur dioxide or any other antioxidants, we remove excess phenolic substances from it. This means that antioxidants are not used in the grape processing technology until the must has been clarified. Phenolic substances in must or mash are oxidised with atmospheric oxygen and settled during coarse lees removal. The sulphur dioxide is used for the first time after juice clarification. In the later stages of maturation and in the final product, wine with a minimum content of phenolic substances is less prone to oxidation (Pokrývková et al., 2020). Phenolic compounds act in wine, among other things, as antioxidants, the main mechanisms being free radical scavenging and metal chelation (Singleton, 1988). The content of total phenolic compounds decreased in our experiment based on the oxygen access and the chosen technology. In Fig. 5, a significant decrease can be seen in not only the variants aged in wooden barrels but also in the variants with fine lees in stainless steel tanks. This decrease in total phenolic substances is explained by their binding to the fine lees. This is supported by the findings of Singleton (1988) and Vasserot et al. (1997), who agr that the interaction between fermentation lees and polyphenols can reduce the amount of free polyphenols in wine. In contrast, the antioxidant activity was significantly higher in the variants trained on yeast lees, as can be seen in Fig. 6.

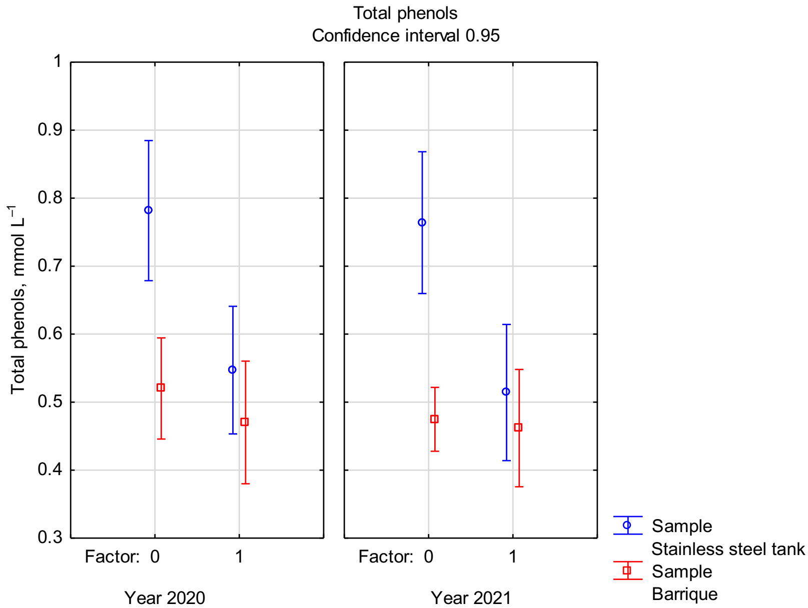

Fig. 5. Content of phenolic compounds. Factor 0 (filtered variant), factor 1 (fine lees)

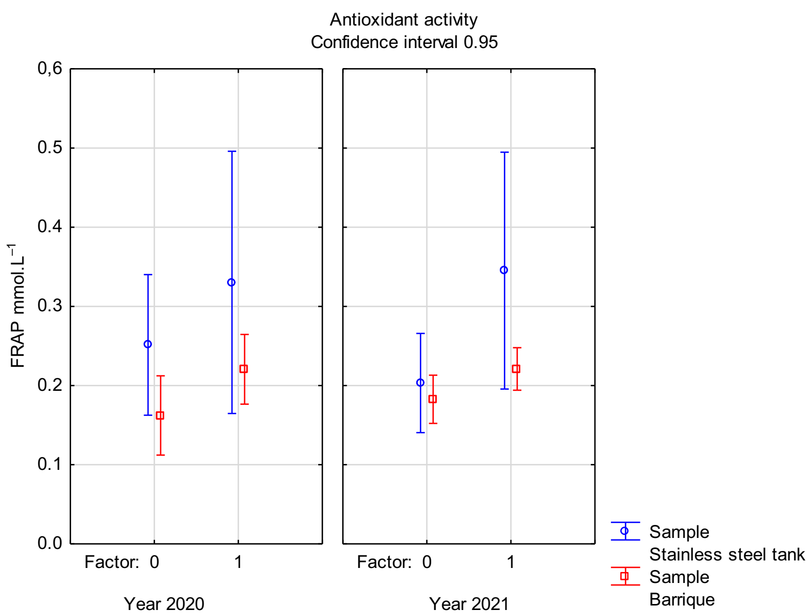

Fig. 6. Antioxidant activity. Factor 0 (filtered variant), factor 1 (fine lees)

4. CONCLUSIONS

The amount of dissolved oxygen in wine changes dynamically during the course of winemaking. During the movement of the wine, whether it is racking, clarification, filtration, or bottling, the wine is exposed to a large amount of oxygen, which, once dissolved, reacts with the components of the wine. From all information and data obtained, we can report that fine lees has a very positive effect on the amount of oxygen in wine, since they have a great ability to absorb dissolved oxygen.

How much oxygen enters the wine and how it is processed depends on many factors: the equipment used, the handling method, the technology chosen, the temperature and chemical composition of the wine. The most significant oxygenation occurs during the filtration and bottling processes; however, it has been found that wines trained on yeast lees can withstand this addition of oxygen very well and that the quality of the wine is not compromised.

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