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Preliminary communication

THE EFFECT OF FINE LEES AS A REDUCING AGENT IN SUR LIE WINES, AGED WITH VARIOUS SULPHUR DIOXIDE CONCENTRATIONS

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During the ageing in barrels, the contact with the fine lees triggers several processes in wine. Lees has a reductive effect by absorbing dissolved oxygen and reducing the amount, which will remain in the wine. At present, minimizing the addition of sulphur dioxide is the trend in all viticultural areas. In this study, the effect of various sulphur dioxide levels was monitored in presence of the lees to determine which dose is appropriate to provide the protection of susceptible white wine against oxidation.

Without SO_2 protection, the rH and redox potential changed slightly, so the level of dissolved oxygen seemed to be controlled during the ageing period by the lees, though the antioxidant effect of lees in itself was not appropriate to protect the polyphenol content from chemical oxidation, which led to considerable browning. With the addition of a lower amount of SO_2 – 40 mg l^{-1}, the lees is already able to protect the white wine samples in all aspects.

Keywords: sur lie, fine lees, reducing agent, polyphenolic compounds

The sur lie method is a special wine making technology, where the contact between the new wine and the fine lees can be taken advantage of. It has a special momentum, namely the battonage, when the wine is stirred periodically in the barrels. This technology originates from France, but nowadays wine makers – in all viticultural areas – use it to widen the white wine assortment. The contact with the fine lees plays an important role in traditional champagne production, too (Fia et al., 2016). From the practical point of view, the robust/full bodied wine, which is properly rich in alcohol and acids, is fit for ageing on lees in oak barrels.

The composition of fine lees is varying. It contains mainly yeast cells, and in much lower amount, tartaric acid and other organic and inorganic matter. The autolysis is known as a slow process that occurs during the wine ageing period (between 12 to 18 months). So the intracellular compounds, enzymes, plasma membrane, etc., are released just approximately after one year (Lavigne et al., 2007; Pérez-Serradilla & Luque de Castro, 2008).

It was shown that the resuspension of the lees by stirring (battonage) during the aging period significantly increased the macromolecular extract of the wine (especially mannoproteins, proteins) beside saturated fatty acids and glutathione. The yeast strain, the turbidity of juice during the fermentation, the contact time and temperature also have effects on the migration of the polysaccharides (Pérez-Serradilla & Luque de Castro, 2008).

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Phenolic substances of the wine cause the bitter and astringent flavour and the browning susceptibility of the wine. The flavonoid phenols have reducing and antioxidant properties, and they are frequently polymerized. These compounds, which originate from grape and also from oak barrels, are linked to mannoproteins from lees, and these complexes enhance the fullness in mouthfeel (Salmon et al., 2000; Landrault et al., 2001; Charpentier, 2010; Gallardo-Chacón et al., 2010). Leskó and co-workers (2011) found that the various enzyme preparations have significant effects on the polyphenolic composition of white wines kept on fine lees.

Earlier studies show that during the ageing there are interaction between oxygen and the lees. Lees in these conditions has a reductive effect by absorbing dissolved oxygen and reducing the amount that will remain in the wine (Fornairon-Bonnefond et al., 2003). There is an empirical basis for the length of contact time desirable to achieve the flavour, and to avoid the wine from having a negative, highly reduced character. Some membrane elements of yeast are in contact with dissolved oxygen (even at a very low concentration), and these compounds, for example ergosterol and other sterols, undergo a mild oxidation, which mechanism is independent from the residual cell viability (Fornairon-Bonnefond & Salmon, 2003). The oxygen consumed by the fine lees during the ageing explains the presence of the lipid peroxides (and other end-products). During the ageing process, reactive groups can get out from the surface of the yeast lees to the wine medium, but the progressive loss of structural biomolecules could reduce the number of reactive groups (Salmon, 2006; Gallardo-Chacón et al., 2010).

The aim of this work was to investigate and describe the reducing impact of the fine lees with various level of sulphur dioxide. The level of protection was established as sufficient, or as the signs of oxidation could be found, like browning of colour and the change in redox potential.

1. Materials and methods

1.1. Materials

The late harvested grape (variety: Chardonnay, vintage: 2015) was processed with reductive technology. The parameters of must and wine treatments were the following: must sulphiting (5 g q^{-1} potassium bisulphit), pectolytic enzymatic treatment (1.5 g q^{-1}), must clarification (10 °C, 24 h), yeast starter (20 g hl^{-1}), fertilizer (20 g hl^{-1}). After the fermentation (approximately 14 days), the base wine was divided into separate units (barrels, capacity: 50 l), and the new wine was sulphited with sulphur levels of 0, 40, and 60 mg l^{-1}. All treatments were set in triplicate. During the ageing period, all samples were stirred twice a week. The independent factors were the various reducing agents, ensured by the presence of fine lees, and the various levels of SO_2, while the dependent factor was the complex maturing of the wine that we followed monthly through the changes of rH, redox potential, acetaldehyde and polyphenol concentrations, and colour.

1.2. Methods of analysis

- Free- and total sulphurous acid concentration, titrimetry (OIV-MA-AS323-04A; OIV, 2016)
- Total acidity (OIV-MA-AS313-01)
- pH (OIV-MA-AS313-15)

- Acetaldehyde (OIV-MA-AS315-01)
- Measurement of the oxidation-reduction potential in wines (OIV-MA-AS2-06)
- Folin-Ciocalteu index- polyphenol concentration (OIV-MA-AS2-10)
- Leukoanthocyanin concentration (Flanzy modified method, 1969)
- Catechin concentration (Rebelein method, 1965)
- Conductivity (OIV-MA-F1-01)
- Chromatic characteristics (OIV-MA-AS2-07B)

1.3. Statistical evaluation

The experimental results were analysed with one-way analysis of variance (ANOVA) and correlation analysis.

2. Results and discussion

The reducing effect of the fine lees was monitored to determine whether it provided sufficient protection from chemical oxidation (as antioxidant, flavour and aroma preservation, and colour stabilization) or not. At the start of ageing, our samples were homogeneous in every aspect except the sulphur level. During the time period of the measurement they changed in analytic parameters because of the different reducing agents used.

After the alcoholic fermentation, the new wine base parameters fulfilled the expectations of sur lie ageing from the perspective of alcohol (14.26% v/v) and sugar concentrations (7.76 g l^{-1}), total acidity (6.7 g l^{-1}), and pH (3.53). Therefore, the samples at the start of the ageing period were appropriate to the sur lie technology, so the changes that we measured monthly were caused mainly by the various reducing properties (and not by the inappropriate wine sample).

Fine lees has been reported as a reducing agent, an antioxidant medium (Salmon et al., 2000; Pérez-Serradilla & Luque de Castro, 2008). At present, minimizing the addition of sulphur dioxide is a trend in all viticultural areas. In presence of the lees, the effect of various sulphur dioxide levels can be monitored, determining which dose is appropriate and how long this protection lasts. Sample no. A.30 (then A.60 and A.90) were protected from chemical oxidation only with the reductive capacity of the fine lees (in these samples the presence of SO_2 came only from the must sulphiting). An average and a lower free sulphur dioxide level – 30 and 15 mg l^{-1} – are represented by the samples B and C under the ageing period. The free sulphur dioxide levels slightly decreased in the measured time period in every sample (Table 1). After 90 days, the amount of free SO_2 was only appropriate to continue the ageing safely in samples C.90. The changes in total SO_2 level meant the amount of sulphite that was oxidized to sulphate as an antioxidant, decreasing the dissolved oxygen level.

The changes in the reducing effect of the lees and SO_2 were measured through the rH (oxidation-reduction level), which did not change significantly during the time studied (Table 1). Neither the redox potential has changed significantly in time, but it was significantly lower in the samples with 60 mg l^{-1} initial SO_2 than in samples with zero initial SO_2 (Table 1). Though in the investigated 90 days considerable difference could not be found, the lees without sulphur dioxide showed slightly less protection. This later could lead to significant changes in redox potential, so the signs of oxidation may be found. From the aspect of SO_2 dosage, even a lower level – 40 mg l^{-1} – could be sufficient in case of short time ageing.

Table 1. The investigated base parameters of the samples with various levels of SO_2

Samples			SO_2 (mg l^{-1})		pH	rH	Redox pot. (mV)	Acetald. (mg l^{-1})
No.	New wine sulphiting	Time (day)	free	total				
A.30	0 mg l^{-1} SO_2		4±1	44±2	3.48±0.03	13.51±0.10	186.8±0.9	69.0±5.0
B.30	40 mg l^{-1} SO_2	30	16±0	80±1	3.55±0.10	13.52±0.48	186.1±0.3	58.0±2.2
C.30	60 mg l^{-1} SO_2		28±2	98±0	3.55±0.10	13.48±0.51	184.6±0.5	60.0±3.0
A.60	0 mg l^{-1} SO_2		4±0	40±3	3.45±0.05	13.32±0.28	186.4±1.0	34.0±4.6
B.60	40 mg l^{-1} SO_2	60	12±1	68±3	3.49±0.07	13.38±0.32	185.9±0.9	44.2±1.0
C.60	60 mg l^{-1} SO_2		20±1	90±1	3.51±0.13	13.36±0.07	184.0±0.6	44.7±2.0
A.90	0 mg l^{-1} SO_2		4±0	42±3	3.50±0.09	13.4±0.17	185.4±0.5	35.8±3.1
B.90	40 mg l^{-1} SO_2	90	12±2	76±2	3.52±0.04	13.41±0.09	185.0±0.1	44.8±1.9
C.90	60 mg l^{-1} SO_2		22±2	92±2	3.53±0.08	13.4±0.40	184.8±0.7	44.1±1.7
SD					n.s.	n.s.	*	*

Values are means and standard deviations of triplicate fermentations; *: significant difference, $P<0.05$; n.s.: not significant difference

The acetaldehyde concentration changed significantly with time in every sample (Table 1). The considerable decrease could be explained by the presence of the lees, more precisely the yeast-derived enzyme, the alcohol dehydrogenase (EC 1.1.1.1). The enzyme activity decreased continuously during the ageing. The acetaldehyde level decreased most (13.8–35.0 mg l^{-1}) between days 30 and 60. From 60 to 90 days the change seemed to stop. This could be attributed to the presence of lees, however the measured period was not long enough to find increasing acetaldehyde level as a result of oxidation. An extended ageing period would be worth examining in further investigations.

Unfortunately, the volatile acidity significantly increased during the investigated period in all samples. The acetic acid level ascended above the organoleptic threshold (0.78–1.05 g l^{-1}). This phenomenon is linked to the presence of acetic acid bacteria, which are obligate aerobe microbes. The limited available oxygen in the barrels was enough for them to grow. Therefore we could draw only limited conclusions, however between the effect of the three different SO_2 levels we could not find significant differences, but in the time there was considerable increase (data not shown).

The presence of total polyphenol-, leukoanthocyanin-, and catechin contents can be considered normal in white wines. The deepening of colour ($A_{420\ nm}$) has to be discussed together with these parameters. The polyphenols are susceptible to oxidation. It can be a non-enzymatic chemical reaction or an enzymatic reaction with polyphenol oxidase (EC 1.10.3.1) (Leskó et al., 2011). In the media the presence of SO_2 could protect the polyphenols as an antioxidant. In the samples with SO_2, the colour and the – not oxidized – polyphenol concentration had not changed considerably under the measured period. These parameters changed significantly in samples without SO_2 protection. In the lack of the antioxidant agent (SO_2), the reducing effect of the lees ought to protect the susceptible polyphenols, which was not sufficient in our samples. The changes of total polyphenol content and of the browning were in strong correlation (r= –0.96607), because the polyphenols are responsible for the

colour of wines (Table 2). Figure 1 and Figure 2 show that the changes were related (the browner colour = higher absorbance is caused by the higher amount of oxidized polyphenols). In samples A.30, A.60, and A.90, the degree of browning – the oxidation of polyphenols – was very quick. The reducing effect of the fine lees was not appropriate at all, the protection from polyphenol oxidation and the colour stabilization was not ensured without SO_2 addition. From the aspect of SO_2 dosage, even a lower level – 40 mg l^{-1} – had appropriate antioxidant effect.

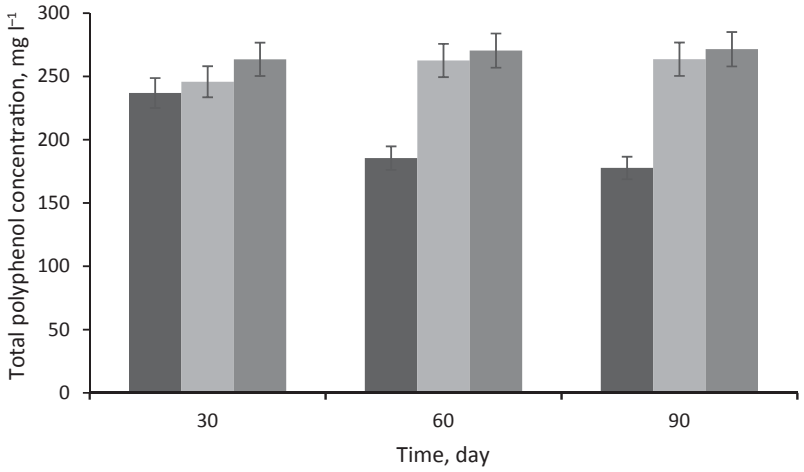

Fig. 1. Changes in total polyphenol content during the measured period
■: 0 mg l^{-1} SO_2; ■: 40 mg l^{-1} SO_2; ■: 60 mg l^{-1} SO_2

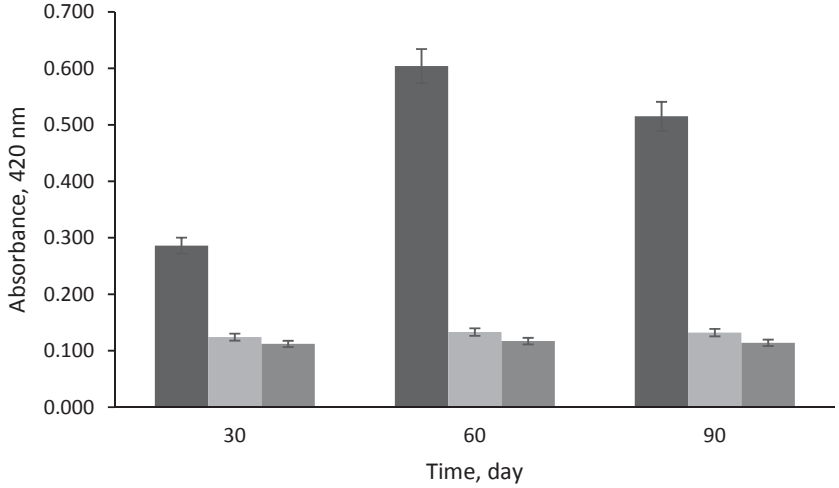

Fig. 2. Changes in the colour – in the brown tone – during the measured period
■: 0 mg l^{-1} SO_2; ■: 40 mg l^{-1} SO_2; ■: 60 mg l^{-1} SO_2

Table 2. The polyphenol content and the colour of the samples with various levels of SO_2

Samples No.	New wine sulphiting	Time (day)	Total polyphenol (mg l^{-1})	Leuko-anthocyanin (mg l^{-1})	Catechin (mg l^{-1})	Absorbance (at 420 nm)
A.30	0 mg l^{-1} SO_2		236.9±5.1	12.5±10.0	70.0±2.5	0.286±0.004
B.30	40 mg l^{-1} SO_2	30	245.8±3.9	24.1±2.9	93.0±8.1	0.124±0.010
C.30	60 mg l^{-1} SO_2		263.5±5.1	26.0±6.6	105.0±3.7	0.112±0.004
A.60	0 mg l^{-1} SO_2		185.5±4.6	26.8±5.4	83.8±1.4	0.604±0.016
B.60	40 mg l^{-1} SO_2	60	262.6±2.8	69.0±3.4	91.8±5.4	0.133±0.004
C.60	60 mg l^{-1} SO_2		270.4±1.7	73.6±5.7	55.9±6.0	0.117±0.011
A.90	0 mg l^{-1} SO_2		177.7±6.1	37.3±1.9	49.9±9.1	0.515±0.020
B.90	40 mg l^{-1} SO_2	90	263.6±3.4	65.9±9.0	81.4±4.6	0.132±0.003
C.90	60 mg l^{-1} SO_2		271.5±1.1	66,0±3.3	90.7±3.2	0.114±0.004
SD			*	*	*	*

Values are means and standard deviations of triplicate fermentations; *: significant difference, $P<0.05$;

3. Conclusions

Summarizing the results, we can conclude that the fine lees has a limited reducing potential to partially protect the new wine from early senescence. Without SO_2 protection the rH and redox potential changed slightly, so the level of dissolved oxygen seemed to be controlled during the ageing period. In contrast to this, the antioxidant effect of lees was not appropriate to defend the polyphenol content from chemical oxidation, which led to considerable browning. In a next experiment it would be worth to measure directly the dissolved oxygen concentration. Under present conditions, the lees with a lower SO_2 presence – 40 mg l^{-1} – already protected the white wine samples in all aspects.

References

Charpentier, C. (2010): Ageing on lees (sur lie) and the use of speciality inactive yeasts during wine fermentation. -in: Reynolds, A.G. (Ed.) *Managing wine quality. Vol. 2. Oenology and wine quality.* Woodhead Publishing, Cambridge, UK, pp. 164–169.

Fia, G., Zanoni, B. & Gori, C. (2016): A new technique for exploitation of wine lees. *Agric. Agric. Sci. Proc., 8,* 748–754.

Flanzy, M., Aubert, S. & Marinos, M. (1969): New technique for determination of leucoanthocyanic tannins. *Ann. Technol. Agric., 1,* 327–328.

Fornairon-Bonnefond, C. & Salmon, J.M. (2003): Impact of oxygen consumption by yeast lees on the autolysis phenomenon during simulation of wine aging on lees. *J. Agr. Food Chem., 51,* 2584–2590.

Fornairon-Bonnefond, C., Aguera, E., Deytieux, C., Sablayrolles, J.M. & Salmon, J.M. (2003): Impact of oxygen addition during enological fermentation on sterol contents in yeast lees and their reactivity towards oxygen. *J. Biosci. Bioeng., 95,* 496–503.

Gallardo-Chacón, J J., Vichi, S., Urpí, P., López-Tamames, E. & Buxaderas, S. (2010): Antioxidant activity of lees cell surface during sparkling wine sur lie aging. *Int. J. Food Microbiol., 143,* 48–53.

Landrault, N., Poucheret, P., Ravel, P., Gasc, F., Cros, G. & Teissedre, P.L. (2001): Antioxidant capacities and phenolics levels of French wines from different varieties. *J. Agr. Food Chem., 49,* 3341–3348.

Lavigne, V., Pons, A. & Duboudieu, D. (2007): Assay of glutathione in must and wines using capillary electrophoresis and laser-induced fluorescence detection. Changes in concentration in dry white wines during alcoholic fermentation and aging. *J. Chromatogr. A, 1139,* 130–135.

Leskó, A., Kállay, M., Nyúl-Pühra, B. & Nyitrai-Sárdy, D. (2011): The change of polyphenolic composition and tyrosol content of the wine as an effect of sur lie method. *Acta Alimentaria, 40* (Suppl.), 79–90.

OIV (2016): International Organization of Vine and Wine: *Methods of analysis*. Aurand, J.M. (Ed.) Available at (http://oiv.int/en/technical-standards-and-documents/methods-analysis/compendium-international-methods-analysis-wines-and-musts-2-vol last accessed 25 March 2016).

Pérez-Serradilla, J.A. & Luque de Castro, M.D. (2008): Role of lees in wine production: A review. *Food Chem., 111*, 447–456.

Rebelein, H. (1965): Contribution to the determination of catechin content in wine (Beitrag zur Bestimmung des Catechingehaltes in Wein). *Deut. Lebensm.-Rundsch.. 61*, 182–183.

Salmon, J.M. (2006): Interactions between yeast, oxygen and polyphenols during alcoholic fermentations: Practical implications. *LWT – Food Sci. Technol., 39,* 959–965.

Salmon, J.M., Fornairon-Bonnefond, C., Mazauric, J.P. & Moutounet, M. (2000): Oxygen consumption by wine lees: impact on lees integrity during wine ageing. *Food Chem., 71,* 519–528.