Monitoring grape ripening by linear sweep voltammetry – Part 1: principle and use to determine the aromatic windows of the harvest

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Summary

Optimum grape maturity is defined according to the style of wine the winemaker wishes to produce. Harvest qualification is therefore of the utmost importance in determining the right date to harvest the grapes, so as to be in the best possible position to produce the desired wine profile.

In addition to traditional monitoring of sugar and acidity concentration, determining the date of sugar loading stop allows us to define harvest windows for producing a given style of wine with a given aroma and balance.

This article describes how linear sweep voltammetry can be used to determine the date of sugar loading stops in a plot. The evolution of the signal obtained from berry crush juices, the same used to determine sugar concentration, is described and the evolution of the signal explained on the basis of the phenomena observed during this phase of ripening. The results show that this technology can be used simply to determine the sugar loading stops in vine plots, and consequently the date windows to make given wine profiles. The use of this indicator to produce style-defined Sauvignon Blanc wines is presented in part 2 of this article.

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Optimum grape maturity is defined according to the style of wine the winemaker wishes to produce. Harvest qualification is therefore of the utmost importance in determining the right date to harvest the grapes, so as to be in the best possible position to produce the desired wine profile.

Traditionally, maturity controls involve analyzing the sugar concentration, pH and total acidity of a plot, and monitoring the average berry weight. The harvest date is chosen on the basis of these parameters, or sometimes on the sugar/acid ratio of the harvest (Ribereau-Gayon *et al.* 1998). However, these analytical parameters cannot be used to determine the aromatic profile of the wine harvested at a given date. In addition, berry tastings can be carried out to determine this aromatic profile, but these are tedious if achieved on a large number of plots.

Deloire (2011) has furthermore described the concept of vine sugar loading: the quantity of sugar per grape berry increases (active loading phase) from veraison onwards until it reaches a plateau, which marks sugar loading stops. From this point, the aromatic profile of the grapes evolves through different types evoking, for example for red grape varieties, red fruit (strawberry/cherry) to black fruit (blackberry/plum). Suklje *et al* (2019) demonstrated on Shiraz wines that these aromatic evolutions were due to different aroma compositions, particularly in fermentative esters. This observation was also made on Cabernet Sauvignon and Shiraz wines by Antalick *et al*. (2021), who further demonstrated that these windows for producing defined wine styles appeared in a temporally reproducible manner after sugar loading stop for a given grape variety, irrespective of vineyard location, mesoclimate experienced by the grapes or vintage.

In addition, Hastoy *et al* (2019) monitored grape ripening during the sugar loading phase using linear sweep voltammetry on berry crush juices during maturity controls. This work showed that the voltammetric signal decreases during the sugar-loading phase until it reaches a signal minimum, which appears to be concomitant with sugar loading stop.

The aim of this study is to understand the origin of the drop in voltammetric signal until a signal minimum is reached at the end of active sugar loading of a plot. First, a molecular origin was sought by evaluating the voltammetric signal of molecules such as sucrose or malic acid. Second, the phenomena occurring during the sugar loading phase (dilution, increase in the quantity of sugar per berry) and after this phase (concentration) were recreated on a solution of green tea phenolic compounds, leading to the hypothesis that the drop in signal observed is linked jointly to the impact of increasing sugar concentration on the intensity-potential curves of the phenolic compounds in the pulp, and to the dilution of the latter due to the increase in berry volume.

Material and method

Linear sweep voltammetry analyses (Polyscan potentiostat, WQS, Vinventions) were carried out in triplicate on printed electrodes (WQS Vinventions, carbon working electrode, Ag/AgCl reference) by applying a potential ramp from 0 to 1200 mV at a scan speed of 10mV/s . Each intensity-potential curve was characterized by calculating the Maturox index, a linear combination of areas under the curve between 400 and 1000mV.

Plot ripening was monitored weekly on samples of 200 berries taken from both sides of the row from mid-veraison to harvest. The 200 berry samples were manually pressed in a plastic bag. The resulting juice containing mainly berry pulp components was analyzed by linear sweep voltammetry as described above.

Solutions of malic acid at 20g/L, sucrose at 200g/L, glucose + fructose (equimolar) at 200 g/L and diammonium phosphate (200 mg/L) in a solution of KCl 0.05M with pH adjusted to 3.20 by 1N HCl were analyzed by voltammetry under the conditions described above.

Finally, to reproduce the effect of ripening (increase in berry volume, increase in sugar concentration as detailed below), a green tea bag (Betjeman and Barton®) was infused for 5 min in 250 mL of water previously heated to 100°C to obtain a solution of phenolic compounds, mainly a flavan-3-ol monomer called epigallocatechin gallate. After cooling to room temperature, several solutions were prepared from the green tea: 20 and 50% dilutions with distilled water, solutions with the addition of an equimolar mixture of glucose/fructose at 100, 170 and 210 g/L to the undiluted tea, at 100 and 210 g/L to the solution diluted to 20% with water, and at 170 g/L to the solution diluted to 50%.

Results and discussion:

Since 2015, weekly monitoring of ripening by linear sweep voltammetry has been carried out on plots of red and white grape varieties, from the end of veraison to harvest. These follow-ups show an overall decrease in intensity-potential curves until a certain date, close to the sugar loading stop according to Hastoy *et al.* (2019), after which the signal stabilizes or increases again. Figure 1 shows an example of the monitoring of a Mourvèdre plot: the intensity-potential curves of samples taken two weeks before sugar loading stop (determined according to the method described by Deloire 2011), at the time of stop and one week after stop are shown. The shapes of the intensity-potential curves remain similar over time (Figure 1). The Maturox index, calculated as a linear combination of areas under the curve, is a numerical indicator of overall signal intensity. In concrete terms, it decreases to a minimum and then stagnates or increases again, making it possible to time the sugar loading stop in a plot, and thus to predict the harvest dates best suited for producing a given style of wine.

During the active sugar loading phase, several phenomena take place in the grape berries:

- Increase in berry volume induces dilutions of compounds which quantity per berry is stable during this phase: tartaric acid, phenolic compounds such as hydroxycinnamic acids or tannins, for example (Teixera *et al.* 2013).
- Increase in the amount of sugar per berry (glucose + fructose from sucrose) and in sugar concentration
- pH increase
- Decrease in total acidity, in particular due to malic acid degradation
- Decrease in ammonium ions per berry
- Increase in amount of anthocyanins per berry (Teixera *et al*. 2013).

Beyond sugar loading stop, increases in sugar concentration are linked to berry transpiration and shriveling, resulting in berry volume losses (Deloire *et al.* 2021).

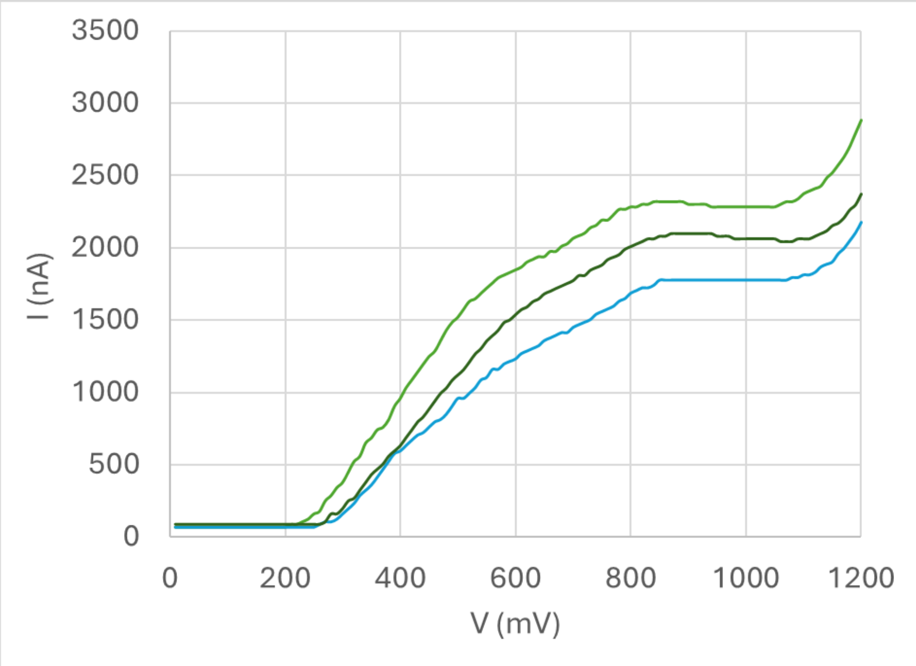

Figure 1: Intensity-potential curves recorded on the juice of 200 berries sampled weekly during ripening in a Mourvèdre plot. Light green: curve from August 12th, 2 weeks before sugar loading stop. Blue: curve from August 26th, date of sugar loading stop. Dark green: curve from September 2nd, one week after sugar loading stop of the plot. Sugar loading stop was determined according to the method described by Deloire (2011).

As a first step, the linear sweep voltammetry signal of various molecules was recorded at their maximum concentration during ripening: sucrose (200g/L), glucose + fructose in equimolar mixture (200 g/L - not shown here), ammonium diphosphate (200 mg/L), L-malic acid (20 g/L). None of these molecules showed a significant signal (Figure 2), especially between 400 and 1000 mV, the potential range considered for calculating the Maturox index for monitoring ripening as described above. The evolution of their concentration during ripening alone cannot therefore explain the changes observed in the intensity-potential curves as described above. The same applies to pH (not shown here), which increase generates slight shifts of the curve on the potential scale, but nothing in common with the signal decreases described above.

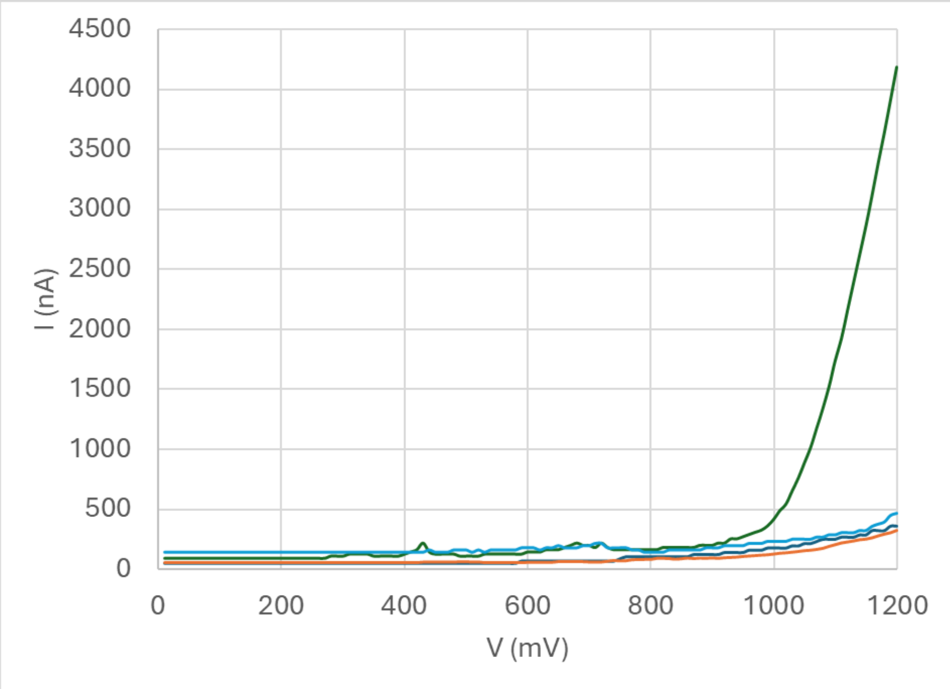

Figure 2: Intensity-potential curves for solutions of sucrose at 200g/L (dark blue), L-malic acid at 20g/L (orange), and diammonium phosphate (200 mg/L - green) in a solution of KCl 0.05M at pH 3.20 (light blue).

It has also been described that the signal obtained on carbon electrodes such as those used in these tests is due to the oxidation of phenolic compounds (Ugliano *et al*. 2019, Kilmartin *et al.* 2001 and 2002). The berry crush juices analyzed here contain mainly pulp-derived compounds, i.e. hydroxycinnamic acids and monomeric flavanols.

As hydroxycinnamic acids and flavan-3-ol monomers (Teixeira *et al*. 2013) are synthesized before veraison, their quantity per berry remains stable during sugar loading, but their concentration decreases as berry volume increases.

In order to reproduce the impact of dilution and increasing sugar concentration in ripening berry crush juices, a green tea solution was chosen as a model solution, as it is rich in monomeric phenolic compounds (epigallocatechin gallate, EGCG), and contains no glucose/fructose or sucrose, unlike grape juice, nor alcohol, unlike wine. It is hypothesized that green tea phenolics behave in a similar way to grape pulp ones in linear sweep voltammetry.

The green tea simulates the crushed juice obtained at the end of veraison, when the sugar content per berry is still low. To represent a berry during active sugar loading phase (20% increase in volume, from 1 mL to 1.2 mL, for example), the green tea was diluted to 20% and 100 g/L sugar (glucose + fructose equimolar) was added. To simulate crushing juice at the end of sugar loading, considering an average berry volume increase of 50% since the end of veraison (from 1 to 1.5mL, for example), green tea was diluted to 50% and 170g/L sugar were added (Shahood *et al*. 2020, Antalick *et al*. 2021, Deloire *et al.* 2021). Finally, to simulate the effect of shriveling by around 20% after sugar loading stop (berry decreasing from 1.5 mL to 1.2 mL, for example), the initial green tea was diluted to 20% (equivalent to a 20% concentration phenomenon on the 50% diluted solution) and 210 g/L sugar were added (average concentration at harvest date).

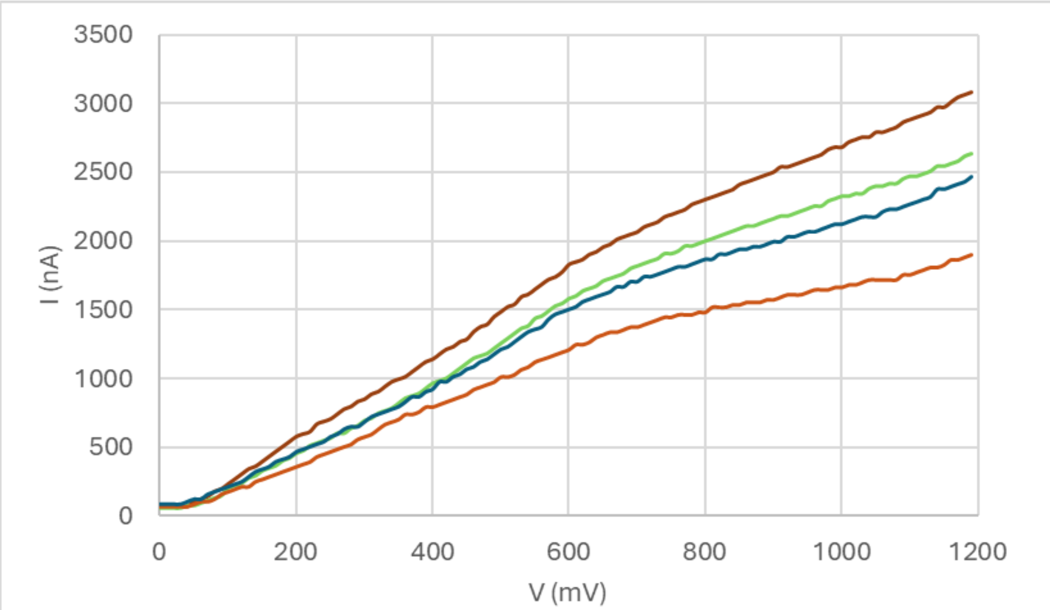

Figure 3: Intensity-potential curves for solutions of undiluted green tea (brown) representing berry crush juice at the end of veraison, green tea diluted to 20% with 100 g/L equimolar glucose + fructose added (green) representing juice during sugar loading, green tea diluted to 50% with 170 g/L equimolar glucose + fructose added (orange), representing juice from berries at sugar loading stop, green tea diluted to 20% with 210 g/L equimolar glucose+ fructose added (blue), representing juice from berries at harvest date.

The intensity-potential curves of the solutions (Figure 3) show that the greater the dilution, the greater the drop in signal, similar to the observations made during ripening. The minimum curve is obtained for the solution simulating berry juice at the end of sugar loading (50% green tea dilution + 170 g/L equimolar glucose + fructose). However, the green tea solution diluted to 20% and containing 210 g/L sugar has a weaker signal than the one diluted to 20% and containing 100 g/L sugar, suggesting a cumulative impact of the two factors on the signal drop. Analysis and calculation of the Maturox (index evaluating the area under the curve of intensity-potential recordings) of tea solutions diluted to 20% and 50% respectively without added sugar, and undiluted green tea with 100, 170 and 200 g/L confirm that the effects of dilution and sugar addition are additive (Table 1). Similar results were also obtained (not shown here) using sucrose (sugar loaded in the grape berry before enzymatic hydrolysis to glucose + fructose) instead of the glucose/fructose mixture. The impact of saccharides on the antioxidant activity of monomeric phenolic compounds measured by $ABTS^{+\bullet}$, $DPPH^{\bullet}$ or FRAP assays has already been reported (Kopjar *et al*. 2016, Peinado *et al*. 2010, Katz *et al*. 2020), as well as non-covalent interactions between phenolic compounds and polysaccharides (Zhang *et al* 2013). These interactions could be at the origin of the formation of supramolecular complexes between phenolic compounds and saccharides resulting in a weaker response in linear sweep voltammetry compared to the signal from phenolic compounds alone.

Solution	Comment	MaturOx (arbitrary unit)	Signal loss relative to tea (delta Maturox - arbitrary unit)	Expected theoretical total (cumulative effect of dilution and addition of sugar)
Undiluted tea	Start of sugar loading	828	-	
Diluted tea 20%+ 100g/L glucose+fructose	Sugar loading in progress	680	-148	-174
Diluted tea 50%+ 170g/L glucose+fructose	Sugar loading stop	492	-336	-306
Diluted tea 20%+ 210g/L glucose+fructose	Shriveling after sugar loading stop (harvest)	604	-224	-259
Tea 50% diluted	Dilution effect only	620	-208	
Tea 20% diluted	Dilution effect only	700	-128	
Tea - 100g/L glucose+fructose	Glucose + fructose effect only	781	-47	
Tea - 170g/L glucose+fructose	Glucose + fructose effect only	730	-98	
Tea - 210g/L glucose+fructose	Glucose + fructose effect only	697	-131	

The evolution of the Maturox index obtained on the tea solutions is similar to what is classically obtained during berry ripening. For example, it has been put into perspective of the monitoring of a plot of Mourvèdre in terms of Maturox and quantity of sugar per berry, which shows a minimum at the 3rd monitoring point, concomitant with the stabilization of the quantity of sugar per berry (figure 4).

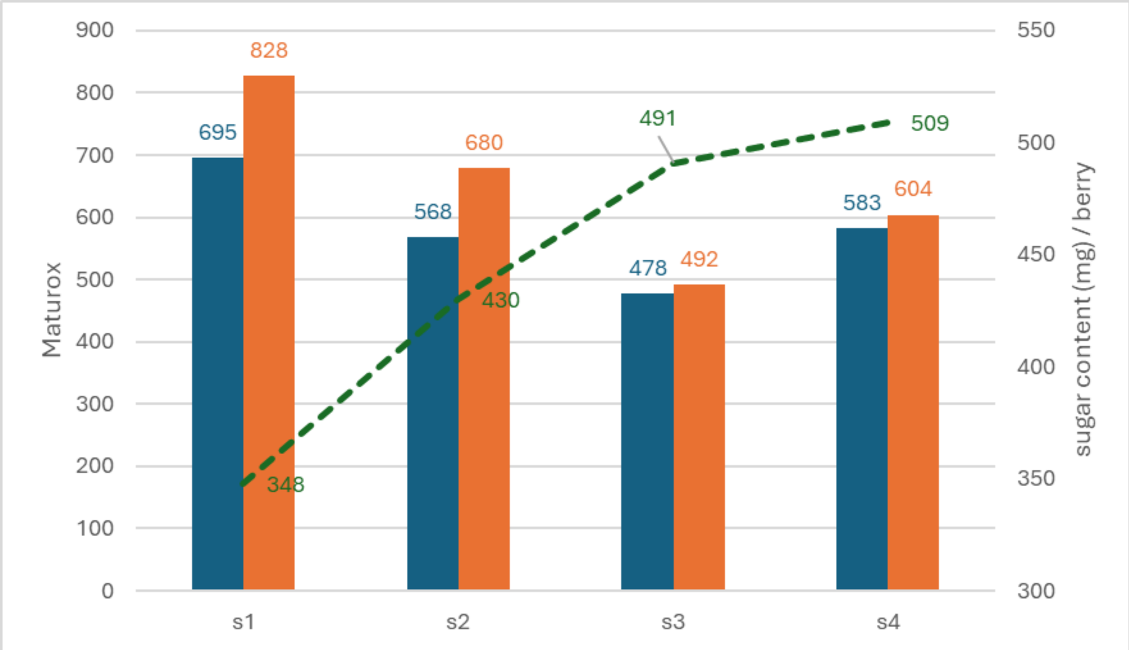

Figure 4: Evolution of the Maturox index of weekly samples from a plot of Mourvèdre during ripening (blue) and of the quantity of sugar per berry (green) from these samples. Maturox (orange) of green tea solutions mimicking the phenomena observed during ripening (figure 3 and table 1).

Considering the results of these trials, the decrease in intensity-potential curves during the sugar loading phase, reaching a minimum when loading stops, seems logical. The phenomenon is based on the evolution of the intensity-potential curves of the phenolic compounds present in the berry pulp during ripening.

During the active loading phase, the sugar concentration in the berries increases. Interactions between sugar and phenolic compounds lead to a drop in the intensity-potential curves for phenolic compounds: the higher the sugar concentration, the lower the signal at a given phenolic compound concentration. In addition, the increase in berry volume leads to a dilution of phenolic compounds in the pulp (biosynthesized before veraison), and therefore to a drop in their voltammetric signal. During this phase, the two phenomena are generally concomitant, leading to a drop in intensity-potential curves. Active sugar loading while berry volume is not increasing also leads to a drop in signal. On the other hand, it is highly unlikely that phenolic compound concentration will continue to decrease as berry volume increases without active sugar loading (McCarthy and Coombes 2008): it therefore seems inconceivable that the signal will decrease without active sugar loading.

From the sugar loading stop, the current-potential curve can :

- evolve little (stable Maturox), if berry volume does not decrease (no shriveling) and sugar concentration changes little (no active loading, no concentration due to shriveling).

- increase (Maturox increasing), if berry volume decreases (back flow / shriveling - Deloire 2021) leading to an increase in phenolic and sugar concentration. In this case, the signal drop linked to the increase in sugar concentration is counterbalanced by the effect of the increase in phenolic compounds. From the results of the tea measurements (Table 3), it appears that during shriveling (20% in the example, from 170 to 210 g/L sugar), the increase in phenolic compound concentration has a greater impact (increase of 80 Maturox units) than the impact of sugar concentration (decrease of 30 Maturox units). Although the effects of the two phenomena are opposite, the resulting signal is a priori higher.

As a result, the minimum signal is necessarily obtained at the moment when active sugar loading stops, and the signal may then remain stable or increase. Linear sweep voltammetry can therefore be used to pinpoint the moment when sugar loading stops in vineyard plots, and consequently to identify the date windows to obtain a given profile

Conclusion:

To master the vinification of defined wine profiles, grapes must be harvested at a date precisely determined in relation to sugar loading stops, as suggested by Deloire *et al.* (2011). Ripening monitoring by linear sweep voltammetry has been put forward to enable determination of this date (Hastoy *et al*. 2019) when a minimum signal is obtained. The present study has highlighted the phenomena underlying the drop in intensity-potential curves during grape ripening. This decrease seems to be linked to the lower concentration of phenolic compounds in the pulp due to the increase in berry volume, coupled with the impact of the increase in sugar concentration on the signal of these same compounds. The use of this indicator to produce style-defined Sauvignon Blanc wines is presented in Part 2 of this article (Brenon *et al.* 2025). This low-juice method could be used in berry-by-berry physiological studies, as recommended by Shahood *et al.* (2020).

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References:

Antalick G., Šuklje K., Blackman J.W., Schmidtke L.M., Deloire A. Performing sequential harvests based on berry sugar accumulation (mg/berry) to obtain specific wine sensory profiles OENO One, 2021, 55 (2).

Brenon E., Morin S., Pascal C. Monitoring grape ripening by linear scanning voltammetry – Part 2: Obtaining wines of Sauvignon blanc with predefined styles . Infowine, 2025.

Deloire A., The concept of berry sugar loading. January 2011, WyneLand

Deloire, A. The concept of berry sugar loading, Wineland Magazine, 2011.

Deloire A., Rogiers S., Šuklje K., Antalick G., Zeyu X., Pellegrino A. Grapevine berry shrivelling, water loss and cell death: an increasing challenge for growers in the context of climate change. IVES 2021.

Hastoy X., Marquier S., Blanc G., Lagarde Pascal C., Use of linear scanning voltammetry to determine harvest date of Sauvignon blanc plots. Revue des œnologues, 2019, 44-46.

Katz I.H., Nagar E.E., Okun Z., Shpigelman A. The Link between Polyphenol Structure, Antioxidant Capacity and Shelf-Life Stability in the Presence of Fructose and Ascorbic Acid. Molecules, 2020, 25(1): 225.

Kilmartin, P.A.; Zou, H.; Waterhouse, A.L. A Cyclic Voltammetry Method Suitable for Characterizing Antioxidant Properties of Wine and Wine Phenolics. J. Agric. Food Chem. 2001; 49; 1957-1965.

Kilmartin P.A., Zou H, Waterhouse A.L. Correlation of wine phenolic composition versus cyclic voltammetry response, Am. J. Enol. Vitic, 2002, 53, 294-302.

Kopjar M., Lončarić A., Mikulinjak M., Šrajbek Z., Šrajbek M., Pichler A. Evaluation of Antioxidant Interactions of Combined Model Systems of Phenolics in the Presence of Sugars. Natural Products Communication, 2016.

McCarthy M.G., Coombe B.G. Is weight loss in ripening grape berries cv. Shiraz caused by impeded phloem transport? Australian Journal of Grape and Wine Research, 1999, 5, 17-21

Peinado J., Lopez de Lerma N., Peinado R. A. Synergistic antioxidant interaction between sugars and phenolics from a sweet wine. Eur Food Res Technol, 2010, 231, 363-370.

Ribereau Gayon P., Dubourdieu D., Doneche B., Lonvaud A. Traité D'œnologie - 1. Microbiologie du vin. Vinifications. Chapitre 10- le raisin et sa maturation. Ed. Dunod, 1998.

Shahood R., Torregrosa L., Savoi S., Romieu C. First quantitative assessment of growth, sugar accumulation and malate breakdown in a single ripening berry. Oeno one, 2020.

Šuklje, K., Carlin, S., Stanstrup, J., Antalick, G., Blackman, J.W., Meeks, C., Deloire, A., Schmidtke, L.M & Vrhovsek, U. Unravelling wine volatile evolution during Shiraz grape ripening by untargeted HS-SPME-GC × GC-TOFMS. Food Chemistry, 2019, 277, 753-765.

Teixeira A., Eiras-Dias J., Castellarin S.D., Gerós H. Berry Phenolics of Grapevine under Challenging Environments Int J Mol Sci 2013 ,14(9),18711-18739.

Ugliano M, Pascal C, Diéval J-B., Vidal S., Wirt J., Bégrand, S. A new voltametric approach for analyzing polyphenols in white grapes and monitoring pre-fermentation operations; Infowine, 2019.

Zhang H., Yu D., Sun J., Liu X., Jiang L., Guo H., Ren F. Interaction of plant phenols with food macronutrients: characterization and nutritional-physiological consequences. Nutrition Research Review, 2013, 21, 1-15.