1. Electrochemical evaluation of laccase activity using CFMEs and constant potential amperometry

As we show in the main text, CFMEs combined with ABTS and cyclic voltammetry facilitate the electrochemical determination of laccase activity (see Figures 1 and 2). The electrochemical assay based on this combination covers laccase activities of interest in winemaking but was found to underestimate the laccase content of must samples (see Table 1). Therefore, we have investigated if replacing cyclic voltammetry with constant potential amperometry improves the performances of the electrochemical assay.

Figure S1A shows current signals recorded after immersing the CFME in solutions with increasing concentrations of laccase and a constant concentration of ABTS and after setting the potential of the CFME to 0 V (*i.e.* to a potential at which oxidized ABTS is electrochemically reduced). While a small and stable background current was recorded in the absence of laccase, the current increased continuously during the 15 min of the experiment when laccase was present in the solution (because the reaction in between laccase and ABTS provided the electrochemical process with oxidized ABTS). The larger the concentration of laccase was, the faster the current increased.

Figure S1B shows the average calibration curve for laccase activity determination obtained with three different CFMEs. As one can observe, there is an excellent linear relationship ($R^2 > 0.99$) in between the slope characterizing the current increase recorded at 0 V applied potential and the laccase concentration. The sensitivity of the assay using amperometry is about the same as the sensitivity of the assay using cyclic voltammetry. Unfortunately, when tested with three spiked must samples, the assay based on amperometry was found to underestimate the laccase content of must samples (just as the assay based on cyclic voltammetry).

This initial study let as conclude that replacing cyclic voltammetry with amperometry does not improve the accuracy of laccase activity determinations in must samples using CFMEs.

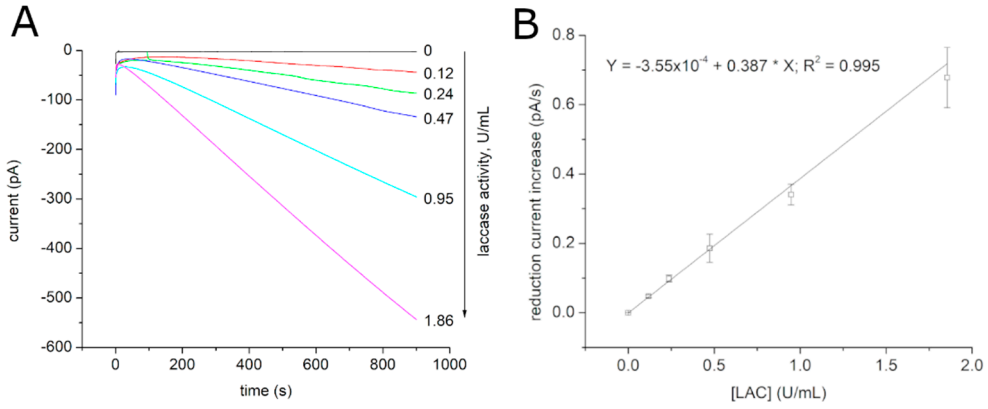


Figure S1. Current signals recorded after immersing the CFME in solutions with increasing concentrations of laccase and a constant concentration of ABTS (0.5 mM) and setting the potential of the CFME to 0 V *vs.* Ag/AgCl (A) and average calibration curve for laccase activity determination obtained with three different CFMEs (B).

2. Voltammetry studies with screen-printed Au electrodes

The possibility of measuring laccase activity electrochemically using ABTS and screen-printed Au electrodes (SPAuEs) was confirmed by cyclic voltammetry studies. A three-electrode setup was used including the SPAuE (catalog number 220 AT from Metrohm DropSens, Spain) as working electrode together with a Ag/AgCL (3M KCL) reference electrode and a Pt wire as counter electrode.

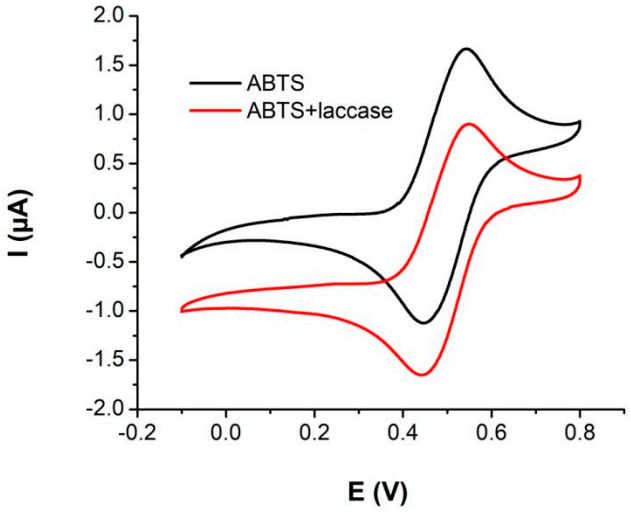


Figure S2. Cyclic voltammograms recorded with a screen-printed Au electrode (SPAuE?) in a solution of 0.5 mM ABTS (made in 0.1 M acetate buffer pH 4.5) in the absence (black) and the presence (red) of 15.8 U/mL laccase. The potential was scanned in the range from -0.1 to +0.8 V at a rate of 5 mV/s.

Figure S2 shows cyclic voltammograms with a pair of well-defined current peaks and emphasizes the quasi-reversible redox couple of ABTS with a formal potential at around 0.5 V corresponding to the one electron transformation (1):

$$ABTS \overset{-e^-}{\rightleftharpoons} ABTS^{\cdot+} \qquad (1)$$

Laccase oxidizes ABTS to $ABTS^{\cdot+}$ in the presence of oxygen. As a higher amount of the oxidized form becomes available, the cathodic current (due to the reduction of $ABTS^{\cdot+}$ back to ABTS) increases significantly and proportionally with the activity of added laccase. Similar to studies with CFMEs, this confirms the possibility to measure laccase activity by determining the cathodic current at 0 V. At positive potentials larger than +0.45 V the electrochemical oxidation of ABTS competes with the enzymatic oxidation by laccase. The magnitude of the anodic current is determined by the relative speeds of the two processes. While the decrease in the anodic current can be also used for analytical purposes for measuring laccase activity, the lack of interferences at 0 V make the measurements at this potential preferable for grape must samples.

3. Comparison of the proposed electrochemical assay with ABTS with spectrophotometry measurements of laccase activity using the same substrate

A total of 14 samples, containing 5 white and 9 red must samples, with laccase activities in the range from 1.0 U/mL to 14.6 U/mL were analyzed in parallel by the proposed electrochemical method and by a spectrophotometry procedure using ABTS as laccase substrate (instead of the syringaldazine used by the classic laccase activity assay developed by Grassin and Dubourdieu [1]).

Spectrophotometric measurements were performed using an Infinite F200 Pro plate reader (from Tecan, Switzerland) equipped with a 405 nm filter. For a direct comparison with the electrochemical assay, 150 µL of ABTS solution (0.5 mM made in 0.1 M acetate buffer pH 4.5) were mixed in the well with 50 µL of laccase-containing sample or standard solution. The absorbance at 405 nm was monitored for 10 minutes and the maximum change in absorbance per minute was correlated with the enzymatic activity. Laccase activity in the must samples was determined based on a calibration plot built using laccase solutions from *Trametes versicolor* of known activities. The activities of the standard laccase solutions were calculated based on their concentration and the specific activity of 78.9 U/mL of the commercial laccase from *Trametes versicolor,* determined by the syringaldazine method of Grassin and Dubourdieu [1], as specified in the main text. The syringaldazine method was taken as reference for expressing laccase activity because the critical levels for enzymatic oxidation in must and wine leading to irreversible damages to organoleptic qualities of wine are expressed in this manner.[1].

The typical calibration curve for the determination of laccase activity by the spectrophotometric, ABTS-based method and the results of the comparison between the spectrophotometric and the electrochemical methods using ABTS are presented in Figure S3 A and B below.

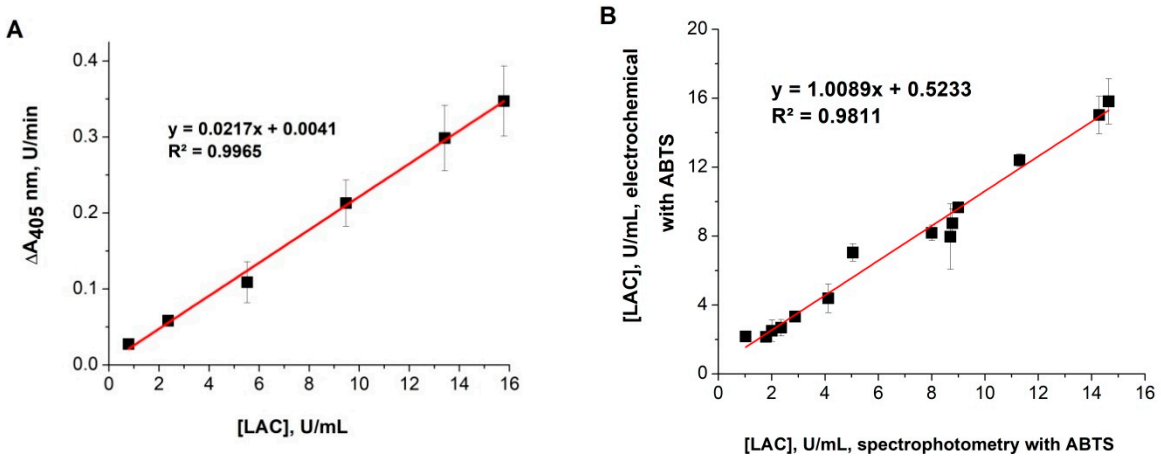


Figure S3. A: Calibration plot for the spectrophotometric method with ABTS, showing the correlation between the change in absorbance per minute at 405 nm and laccase activity. B: Comparison between the proposed electrochemical assay with ABTS and the spectrophotometric method with ABTS.

The results in Figure S3B indicate the quantitative correlation and a good agreement between the spectrophotometric and the electrochemical method, as denoted by the correlation coefficient R^2= 0.9811 and the slope of the plot, close to 1. The value of 1.089 of the slope points to a slight overestimation of laccase activity by the electrochemical assay.

The outcome of the above comparison confirms the hypothesis that the observed differences between the proposed electrochemical assay with ABTS and the spectrophotometric method with syringaldazine (see the main text) are mostly due to the different catalytic efficiencies of laccase with these substrates, the different experimental conditions and the effect of any residual phenolic compounds in PVPP-filtered must on the ability of laccase to convert the two substrates.

With respect to the differences between the enzymatic activity of laccase determined with syringaldazine and ABTS as substrates, one should also note the contradictory literature reports, indicating better affinity with either syringaldazine or ABTS [2-9]. While such variability is explained in part by the different experimental conditions, the lack of unitary conclusions prevents further discussion of our results. More data needs to be gathered in future studies using model solutions of grape must spiked with laccase and relevant phenolics.

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