## *Supplementary information*

**Fast electrochemical measurement of laccase activity for monitoring grapes' infection with *Botrytis cinerea***

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**Evaluation of the sensitivity of the optical assay for laccase activity using individual color channel versus the composite image of the sample**

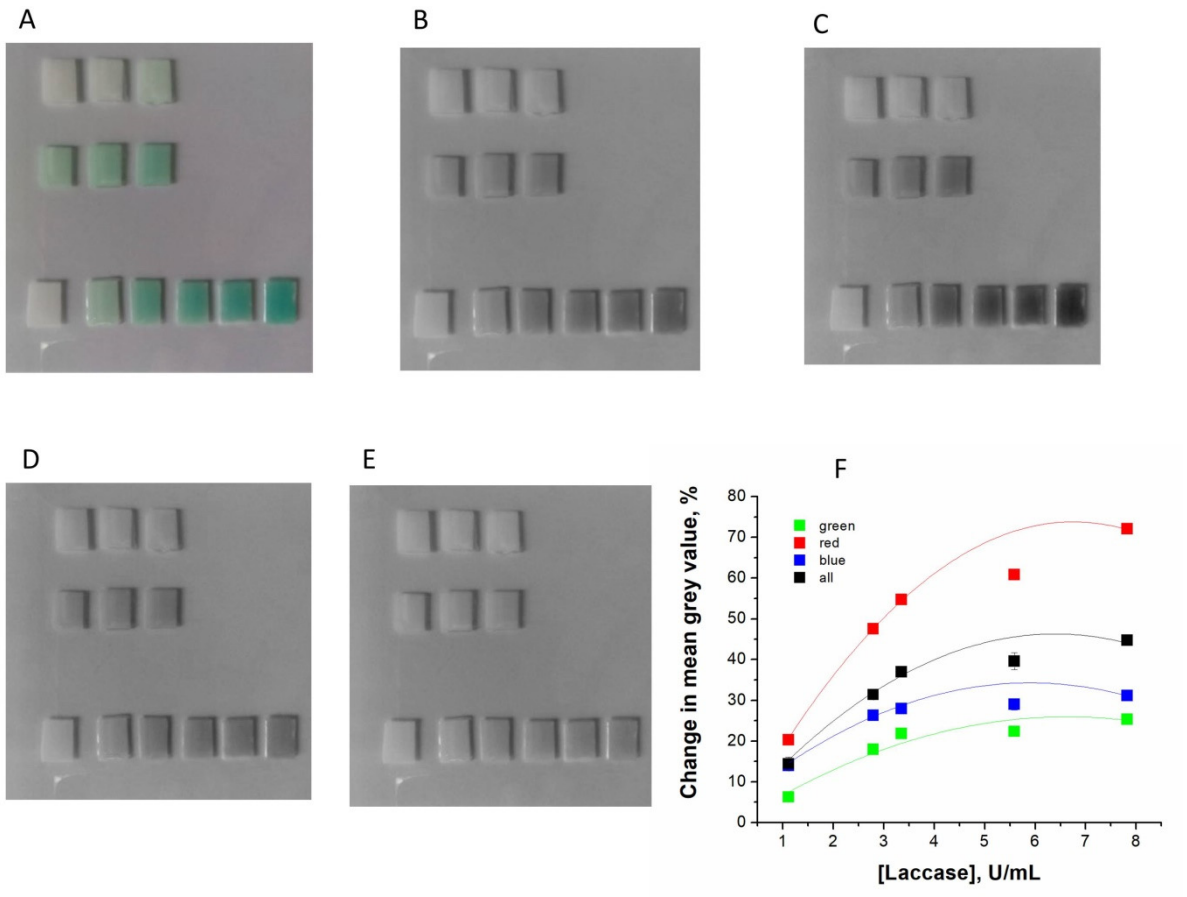



**Figure S1.** A: Full color original picture showing ABTS-based paper sensors after a 10 minutes reaction with fresh white, rosé and red musts (the upper lane). the same musts to which 2.8U/mL laccase was added (middle lane) and laccase solutions of increasing concentrations from 0 to 7.8 U/mL (see Figure 5A in the main text for more explanations). B: the image in A after conversion to a 8 bit greyscale in ImageJ. C: The red (C), blue (D) and

green ( E ) split channels of image shown in A. F: Calibration plots made based on measurements on the red, blue, green channel and the composite image.

**Influence of the type of camera/smartphone on the optical assay of laccase activity using ABTS-impregnated paper.**

To assess the relevance of the cellphone type and camera used for the optical assay of laccase, images were taken with two phones for a series of laccase solutions of increasing concentrations, after 19 minutes of reaction with the paper sensors. A Huawei P Smart 2018 model FIX-LX1 smartphone, equipped with dual camera: a back camera of 13 MP+2MP, used to take the pictures of the sensors and a 8 MP front camera and a Xiaomi 9T Pro, model M1903F11G, equipped with a Sony camera of 48 MP were used in these tests. The images were taken in ambient light with the flash off, from similar relative distance to the paper sensors and included controls (paper reacted with buffer without laccase). In the first series of images (A and B) the pictures were taken at set exposure of 1.3, while in a second series of pictures the "Autoexposure ON" feature was selected. As shown in Figure S2, there are differences due to the different exposure and phone type. The bias due to the phone type is obvious from the calibration plot in Figure S2E. Nonetheless, as long as controls are included in the image (such as the paper sensor on the left which was reacted with buffer solution without laccase) and as long as the photo of the must samples is taken together with that a series of laccase solutions of known activity to build a calibration plot, the color interpretation of the sample based on such calibration is accurate.

The influence of the exposure, lighting, distance and the bias due to the smartphone model need to be considered if developing a specific software application for the color interpretation of the paper sensors for laccase activity.

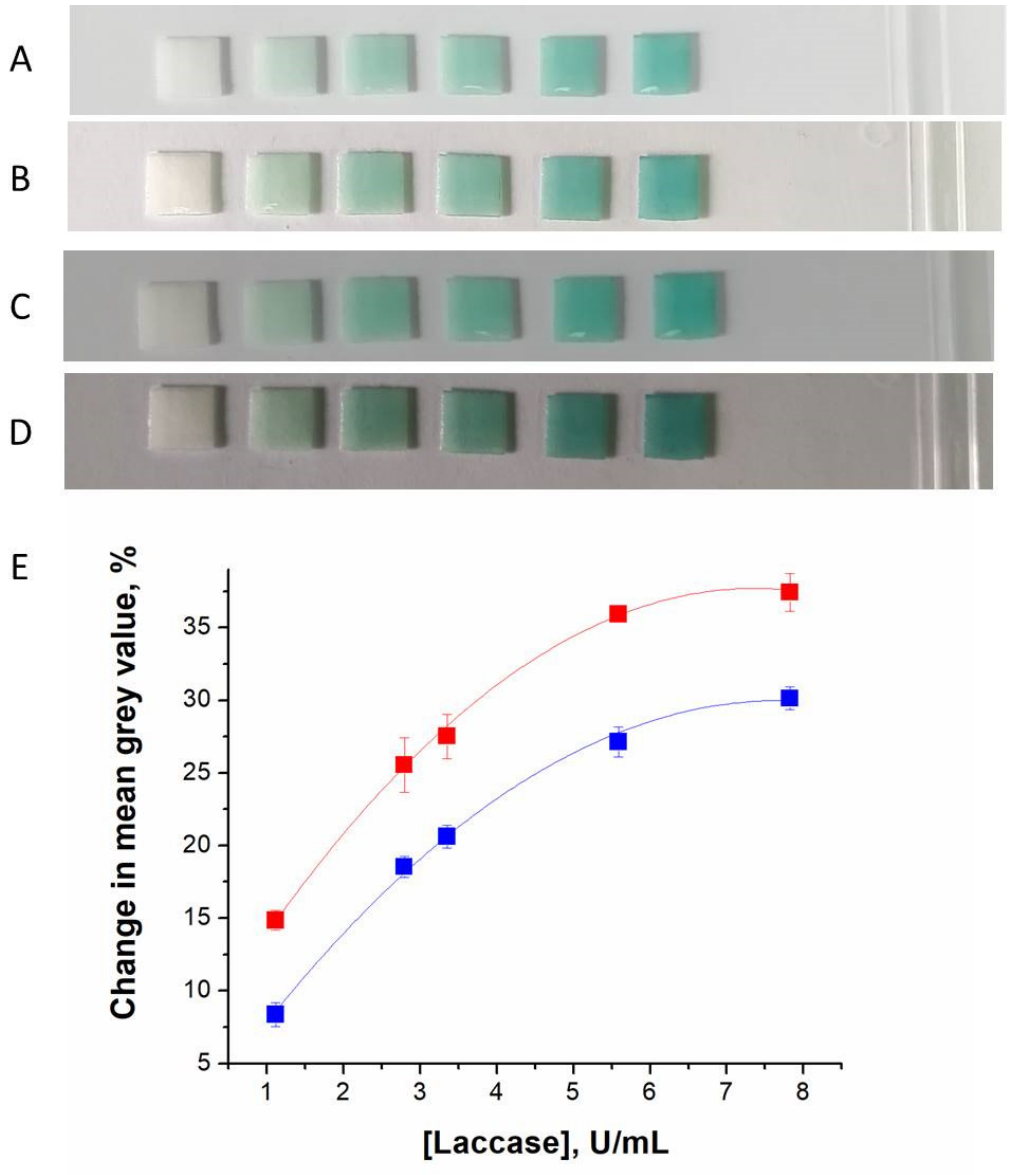



**Figure S2.** A-D. Images of a series of ABTS-based paper sensors reacted for 19 minutes with a series of laccase solutions in 0.1 M acetate buffer pH 4.5 of increasing enzymatic activities, from left to right: 0 (buffer), 1.1, 2.8, 3.4, 5.6 and 7.8 U/mL. A, C: Photos taken with a Xiaomi 9T Pro phone. B, D: Photos taken with a Huawei P Smart 2018 phone. A, B: photos taken at a set exposure of 1.3. C, D: photos taken with with "Autoexposure On" feature enabled on the smartphones. E: Calibration plots for laccase activity based on the photos of the paper sensors reacted with laccase solutions, shown in A and B. The change in mean grey value is calculated as the difference of the mean grey value of the sensor reacted with laccase compared to the control sensor with no laccase (on the left), divided by the mean grey value of the control sensor.

**Applicability of the paper-assisted electrochemical assay for the evaluation of laccase activity of musts**

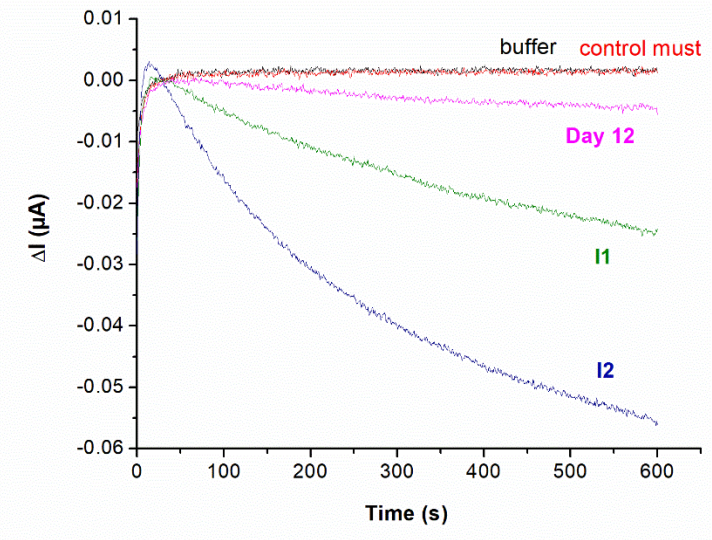



**Figure S3**. Current versus time plots at 0V vs Ag/AgCl for a red must from fresh Tempranillo grapes (control), at 12 days after the inoculation with spores of *Botrytis cinerea* and incubation at 20° C and 55% RH and at after 20 days in the same conditions (I1: grapes with 0-25% infection, I2: grapes with 25-50% infection).

**Table S1**. Enzymatic activity of musts spiked with laccase, measured by the electrochemical method.

| Type of grapes | Laccase added in must ( U/mL) | Laccase found (U/mL) | % Recovery |
|---|---|---|---|
| Red | 0 | nd[a] | N/A[b] |
| | 1.5 | 1.54 ± 0.04 | 103.2 ± 2.5 |
| | 3.7 | 4.15 ± 0.29 | 110.9 ± 7.8 |
| | 5.6 | 4.33 ± 0.42 | 77.3 ± 7.4 |
| Rose | 0 | nd | N/A |
| | 1.5 | 1.18 ± 0.18 | 78.6 ± 12.2 |
| | 3.7 | 3.15 ± 0.09 | 84.2 ± 2.3 |
| | 5.6 | 4.95 ± 0.33 | 88.2 ± 5.9 |
| White | 0 | nd | N/A |
| | 1.5 | 1.78 ± 0.46 | 118.9 ± 30.8 |
| | 3.7 | 4.89 ± 0.25 | 130.7 ± 6.7 |
| | 5.6 | 5.0 ± 0.48 | 94.4 ± 8.5 |

a: nd: not detected, i.e., < 0.4 U/mL; b: N/A: not applicable

**Table S2**. The characteristics of the grapes used in artificial inoculation studies with *Botrytis cinerea.*

| Type of grape and variety | **TPI** | **Sugar (g/l)** | **Total acidity (g/l tartaric acid)** | **pH** |
|---|---|---|---|---|
| Red, Tempranillo | 17±0.0 | 187.4 ± 0.0 | 4.8 ±0.0 | 3.52 ± 0.02 |
| White, commercial | 14.2±0.1 | 157.8±0.0 | 4.20±0.0 | 3.60±0.00 |
| Rose, Red Globe | 14.7±0.3 | 147.1±0.0 | 4.58±0.0 | 3.45±0.00 |
| Red, Moldova | 12.4±0.0 | 145.0±0.0 | 8.85±0.0 | 2.83±0.00 |

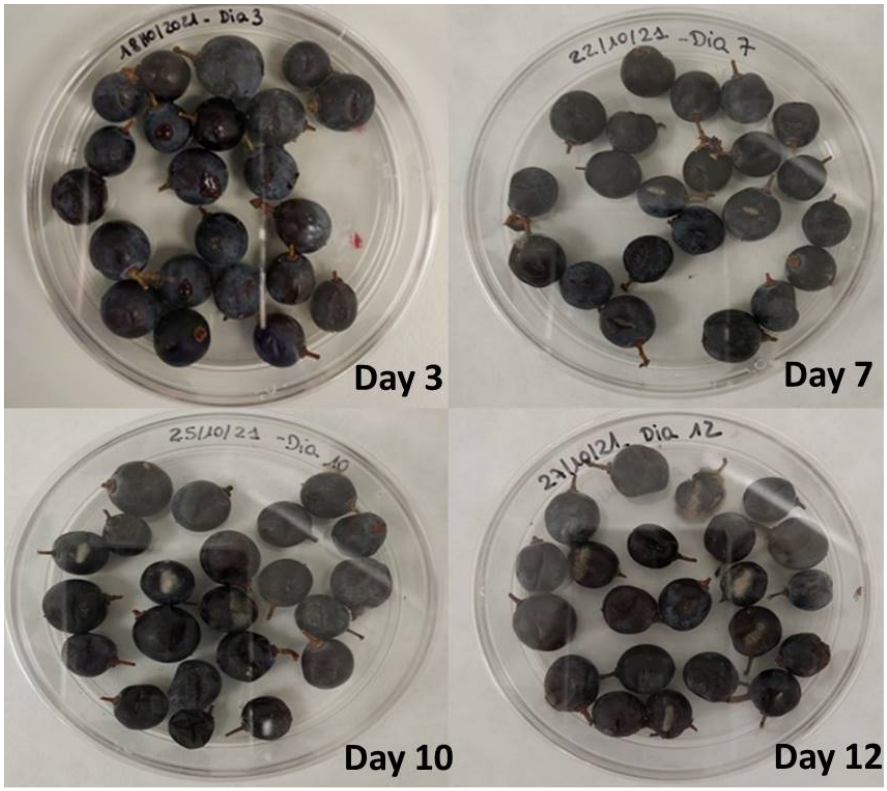



**Figure S4**. Tempranillo grapes after 3, 7, 10 and 12 days of storage at 24° C following the inoculation with *Botrytis cinerea*. After 12 days for the vast majority of berries the infection appears to be localized at the inoculation site.

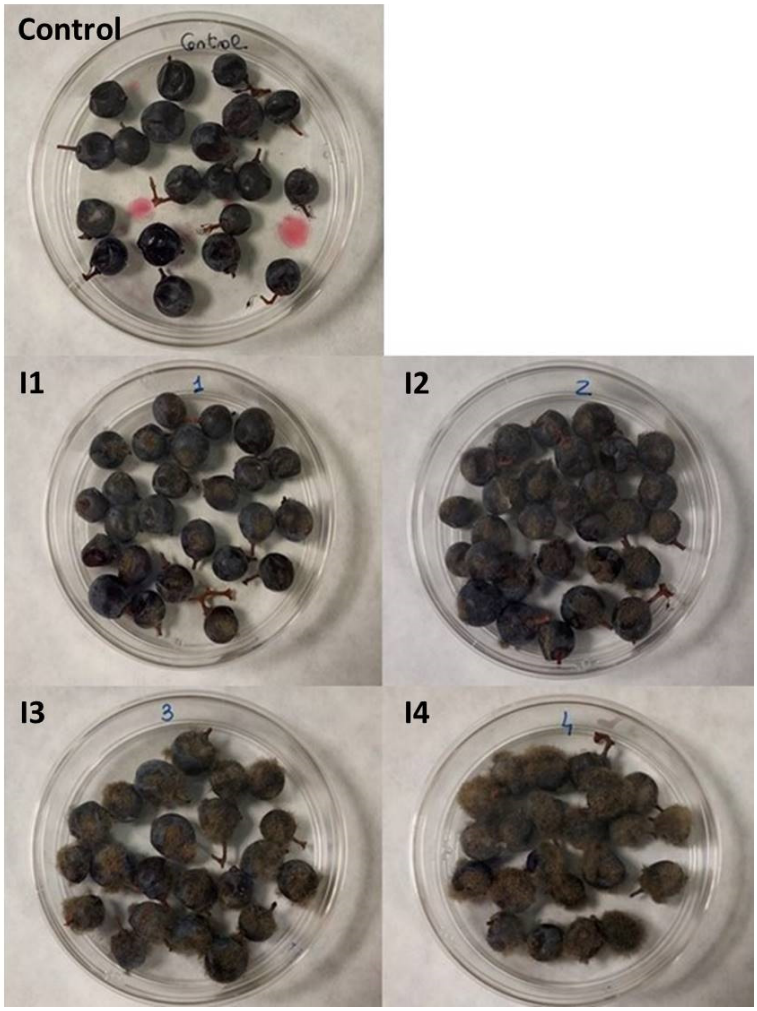



**Figure S5**. Tempranillo grapes artificially infected with *Botrytis cinerea* after 13 days at 24° C and 55 % RH: not infected grapes (control, upper image) and grapes grouped by the infection degree, from I1 to I4. The humidity favored the development of the mycelium which spread and covered up the whole surface of the berries. The variation between the individual berries are quite large, thus their grouping according to the infection degree is more relevant for correlating it with laccase activity.




**Figure S6**. Grapes used in the artificial infection studies with *Botrytis cinérea.* A: White, rosé (Red Globe) and black (Moldova) grapes at the time of inoculation with *Botrytis cinérea*. B: The grapes after 14 days (white) and 17 days (rosé and black), respectively of incubation at 25° C and 90% RH following the inoculation with *Botrytis cinereal.*

**Table S3**. Laccase activity of artificially innoculated Tempranillo grapes determined by the spectrophotometric method with syringaldazine.

| Sample[a] | Average laccase activity (U/ml) | Standard deviation |
|---|---|---|
| D0 | 0.15 | 0.03 |
| D3A | 0.10 | 0.03 |
| D4A | 0.18 | 0.01 |
| D5A | 0.07 | 0.01 |
| D6A | 0.18 | 0.02 |
| D7 A | 0.09 | 0.03 |
| D10 A | 0.18 | 0.02 |
| D12 A | 0.08 | 0.03 |
| D20 I1 A | 4.25 | 0.10 |
| D 20 I2 A | 5.67 | 0.23 |
| D20 I3 A | 7.33 | 0.20 |
| D20 I4 A | 11.30 | 0.04 |
| Control, I0 | 0.00 | 0.00 |
| D13 I1 A | 0.66 | 0.04 |
| D13 I2 A | 1.83 | 0.06 |
| D13 I3 A | 7.33 | 0.23 |
| D13 I4 A | 3.04 | 0.06 |

[a]the sample name code indicates the day after inoculation (e.g., D7 stands for day 7 after the inoculation), infection degree (e.g I3 means berries with the 50-75 % of the surface covered by mycelium) and strain of Botrytis used for inoculation (A in this case).

**The influence of the temperature on the activity of laccase**

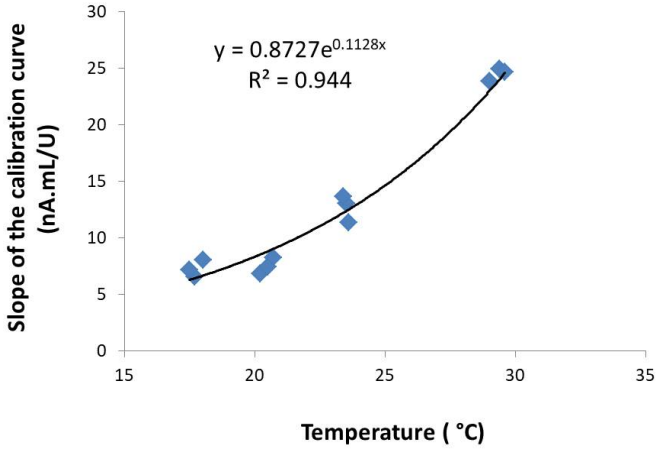



**Figure S7**. Influence of the temperature on the activity of laccase evaluated via the amperometric assay with ABTS in solution [1].

The influence of the temperature on the activity of laccase was evaluated via the previously described amperometric assay with ABTS mixed with the sample in solution [1]. Besides the enzymatic reaction in solution, the temperature also affects the diffusion of the oxidized ABTS to the electrode and the rate of its electrochemical reduction Based on the Arrhenius equation, the rate constant of a chemical reaction is an exponential function of the inverse of temperature.

$k=A \exp(-Ea/RT)$

where Ea is the activation energy, A is a constant specific to each reaction, R is the universal gas constant and T is the temperature. In a simplistic way, in the amperometric assay for the evaluation of laccase activity, the slope of the calibration plot (change in cathodic current versus laccase activity) can be described and fitted as a function of temperature as shown in Figure S5, which is based on measurements in the range from 17° C to 32° C. This function can be used to correct the measured amperometric signals according to the actual temperature in order to provide accurate laccase activity estimations.

**Robustness evaluation of the paper-assisted electrochemical assay**

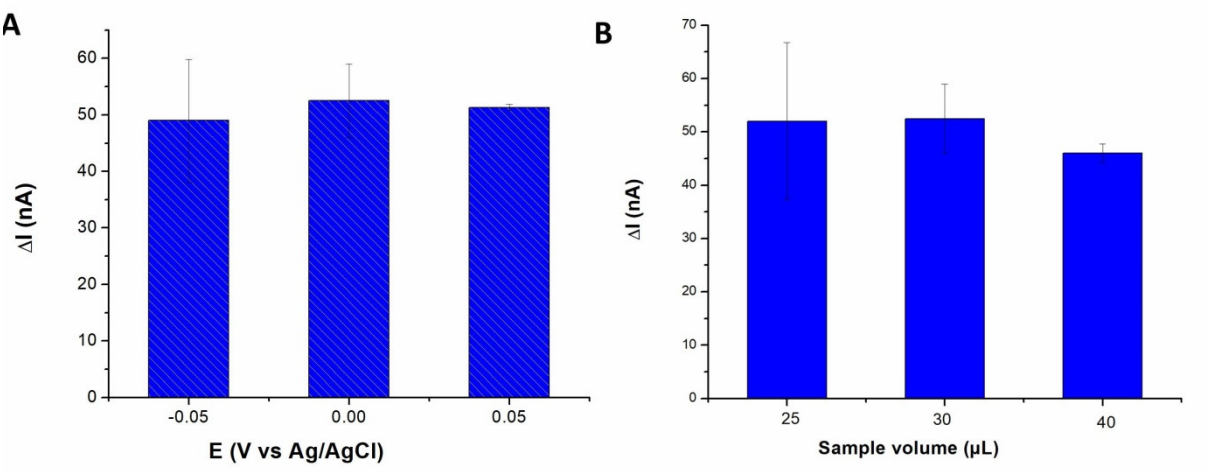



**Figure S8**. The robustness of the proposed method for paper-assisted amperometric assay for the evalution of laccase activity: the influence of the applied potential (A) and the effect of the sample volume (B). The analytical signal consists in the difference between the cathodic current intensity at 300 s and 30 s after applying the potential (0V vs Ag/AgCl). A 2.2U/mL laccase solution in 0.1 M acetate pH 4.5 was tested in the different experimental conditions and the results presented are the mean of 5 replicates.

## References

1. Gáspár, S.; Brinduse, E.; Vasilescu, A. Electrochemical Evaluation of Laccase Activity in Must. *Chemosensors* **2020**, *8*, 126.