Influence of the type of substrate on the laccase browning kinetics

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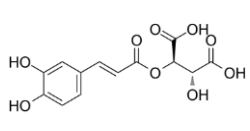
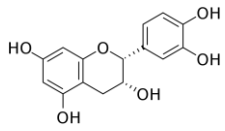
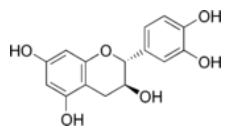
INTRODUCTION

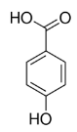
To our knowledge all the studies about laccase kinetics and its inhibition have been performed with substrates and conditions very different from those of real grape juice. Moreover, none of these researches really measure enzymatic browning, since they have not taken into account what happens after the oxidation of o-diphenols in o-diquinones and their subsequent polymerization to form melanins1. For that reason, the aim of this research was to develop a new model to measure the kinetics of browning caused by Botrytis cinerea laccase under conditions much closer to those of grape juice and using the substrates naturally present in it.

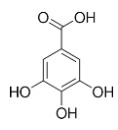
MATERIALS AND METHODS


Caftaric acid


(+)-Catechin

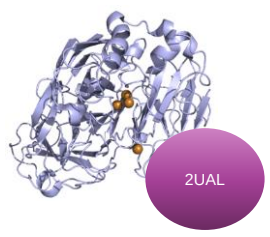

(-)-Epicatechin


4-Hydroxybenzoic acid

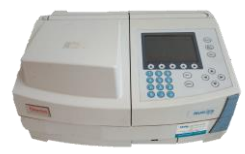

Gallic acid

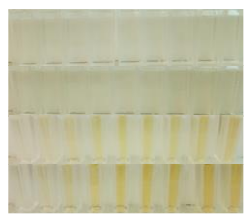
LACCASE BROWNING KINETIC

5 orthophenols with concentrations of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 & 0.8 mM.



MEASURED AT TIME 0, 15, 30 AND 45 MINUTES
The slope of the regression straight line was determined in order to express the intensity of browning.


Absorbance at 420nm



RESULTS AND DISCUSSION

Figure 1: Kinetic of laccase browning (A420) of different orthophenols, from 0.1 to 0.8 mM.

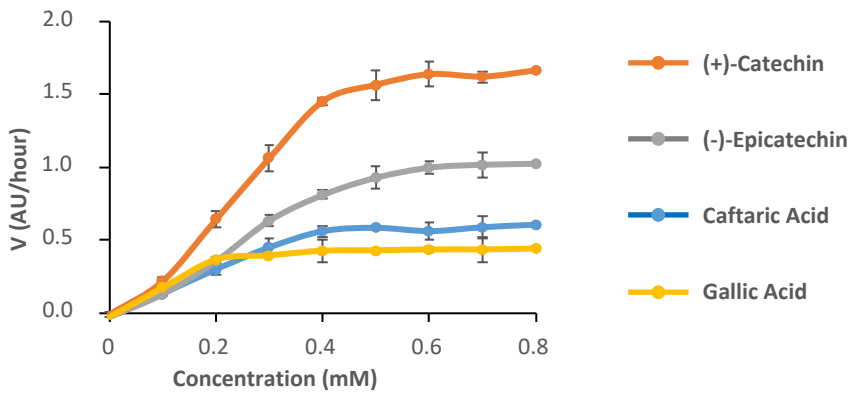


Table1: Effects of different orthophenols on the kinetic constants of laccase.

Substrate	Phenol type	Vmax (AU_{420}/hour)	$K_{0.5}$ (mM)	Hill's number
Caftaric Acid	*o*-Diphenol	0.66 ± 0.04 B	0.173 ± 0.011 B	2.25 ± 0.11 B
(+)-Catechin	o-Diphenol	1.75 ± 0.08 D	0.222 ± 0.015 C	2.65 ± 0.19 C
(-)-Epicatechin	o-Diphenol	1.08 ± 0.06 C	0.223 ± 0.015 C	2.71 ± 0.09 C
Gallic Acid	Triphenol	0.48 ± 0.03 A	0.101 ± 0.008 A	1.74 ± 0.15 A
4-Hydroxybenzoic Acid	Monophenol	nd	nd	nd

- The results indicate that o-diphenols are better substrates for laccase browning than triphenols and that monophenols, or at least 4-hydroxybenzoic acid, do not appear to be reactive.
- Moreover, of the o-diphenols, (+)-catechin showed the greatest browning intensity, followed in decreasing order by (-)-epicatechin and caftaric acid.

CONCLUSION

This research proposes a synthetic model for measuring laccase browning in a matrix close to real grape juice that makes it possible to study how laccase browning acts in the presence of different possible substrates. Further studies are needed to verify the efficiency of the proposed model on other laccase substrates such as anthocyanins, flavonols and proantocyanidins, and also to determine the inhibitory effect toward laccase browning of the most frequently used antioxidants – sulfur dioxide, ascorbic acid and glutathione – and other possible inhibitors of laccase browning such as oenological tannins.

Funding: This work was funded by CICYT (Efecto de las lacasas sobre la sensorialidad, calidad y salubridad de los vinos. Evaluación de la influencia de lacasas sobre el color, la astringencia y la calidad del vino – project RTI2018-095658-B-C33).

Acknowledgements: Authors wants to thank professors Marc Fermaud and Jean Roudet from INRAE, UMR SAVE, Bordeaux Science Agro, ISVV, France for having provided us with the *B. cinerea* strain.

References:

1 Vignault, A.; Gombau, J.; Jourdes, M.; Moine, V.; Canals, J.M.; Fermaud, M.: Roudet, J.; Teissedre, P.L.; Zamora, F. Oenological tannins to prevent Botrytis cinerea damages: kinetics and electrophoresis characterization. Food Chem. 2020, 316 (2020) 126334, doi: 10.1016/j.foodchem.2020.126334

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