Impact of enological tannins on laccase activity

Vignault, A., Pascual, O., Jourdes, M., Moine, V., Fermaud, M., Roudet, J., Canals, J.M., Teissedre, P.L., Zamora, F. *Oeno One*, 2019, 1, 27-38. https://oeno-one.eu/article/view/2361

OENO One
VINE AND WINE OPEN ACCESS JOURNAL

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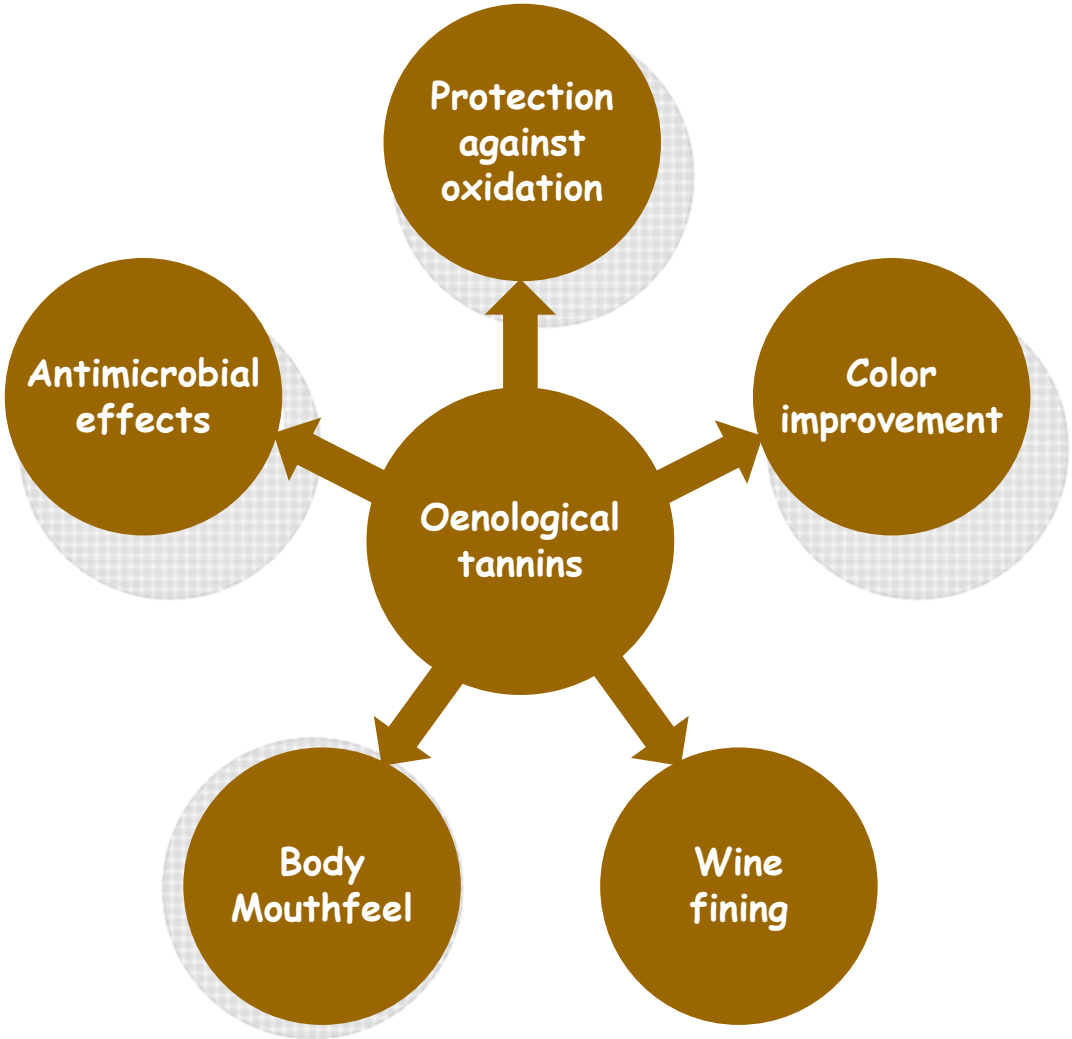
International Organisation of Vine and Wine

- *However we all know that oenological tannins are used for many other aims*



- *Until very recently, the OIV only recognizes the use of oenological tannins as technological aids for wine fining*

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For that reason the OIV working group on oenological tannins has been working on the study of the different functionalities of oenological tannins.

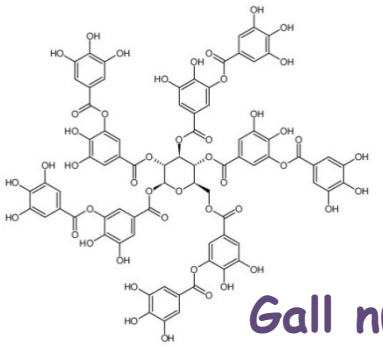
As a fruit of this research the OIV resolutions Oeno-Techno 17-612 and 17-613 have been adopted by the 17TH General Assembly of the OIV recognizing this new functionalities:

- ***Promoting the expression, stabilization and preservation of color, thanks to its effect as copigments.***
  Gombau et al., (2016) BIO Web of Conferences, 7, 02033; Vignault, et al., (2019) BIO Web of Conferences, 12, 02005; Gombau et al., (2019) Oeno One, 3, 531-547; Vignault et al., (2019) Molecules, 24, 1448.
- ***Contributing to the antioxidant protection of components of the must and wine, thanks to their capacity to consume oxygen and their inhibitory action of laccase activity.***
  Navarro et al., (2016) Food Chemistry, 199, 822–827¸ Pascual et al., (2017) Food Chemistry, 234, 26-32; Vignault et al., (2018) Food Chemistry, 268, 210-219; Vignault et al., (2019) Oeno One, 1, 27-38; Vignault et al., (2020) Food Chemistry. Accepted

CLASSIFICATION OF TANNINS

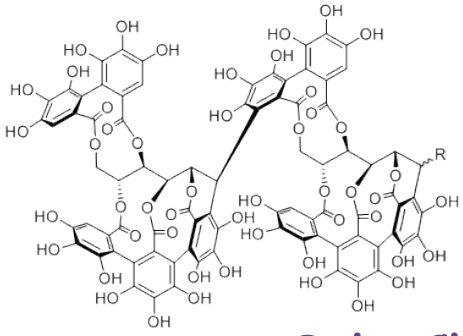
Hydrolysables

Gallotannins



Gall nuts

Ellagitannins

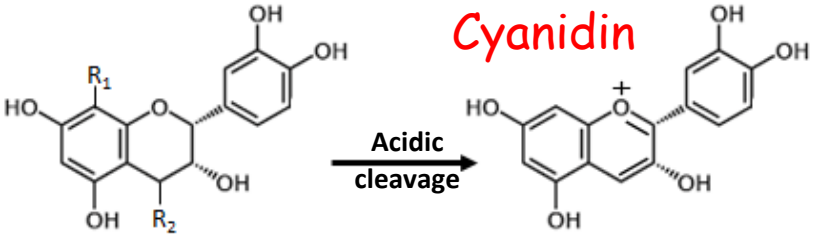


Oak, Chestnut

Condensed tannins or Proanthocyanidins

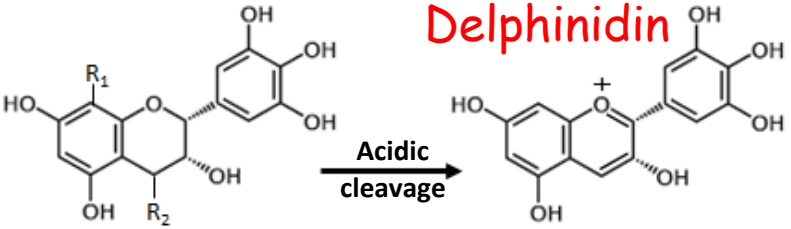
Procyanidins

Grape skins and seeds



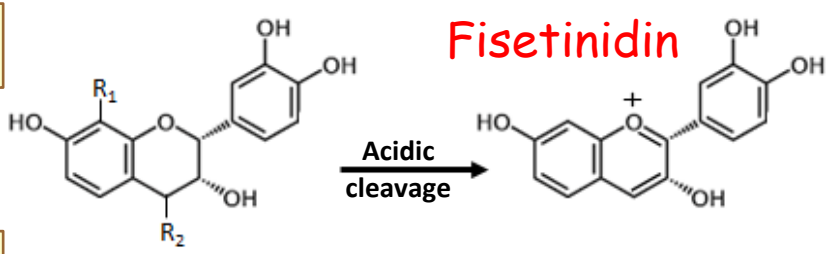
Prodelphinidins

Grape skins



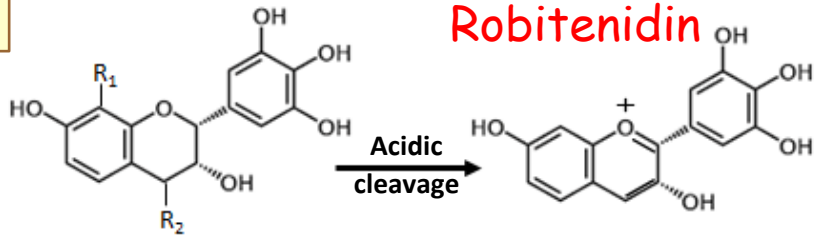
Profisetinidins

Quebracho



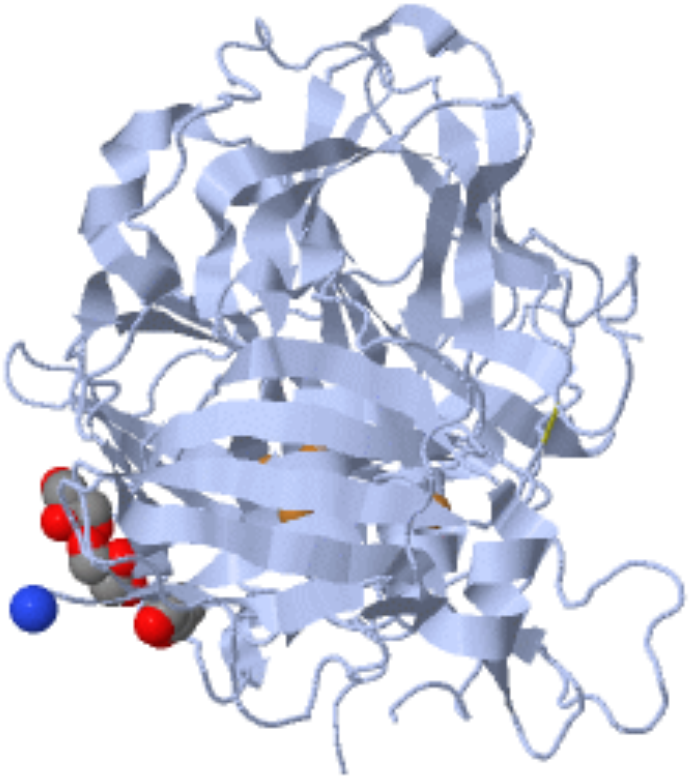
Prorobitenidins

Mimosa



Laccases (EC 1.10.3.2, *p*-diphenol: dioxygen oxidoreductases) are multi-copper proteins that use molecular oxygen to oxidize various aromatic and non-aromatic compounds by a radical-catalyzed reaction mechanism.

Laccase



How can we avoid the damage caused by laccase?

$2\,H_2O$

Laccase
4 x Cu^{+2}
oxidized

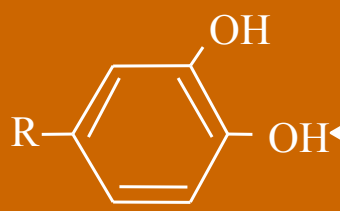
Laccase
4 x Cu^{+1}
reduced

Laccase inhibition
- SO_2
- Oenological tannins

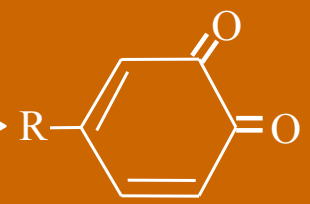
- Inert gases

Avoiding Oxygen
- Ascorbic acid

Glutathione (GSH)

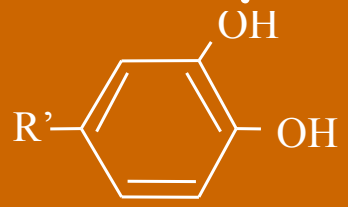


Caftaric Acid

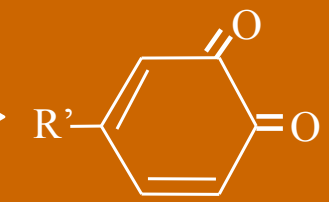


Orthodiquinone of Caftaric Acid

Coupled oxidation



Orthodiphenols:
Anthocyanidins
Flavanols,
GRP, Others

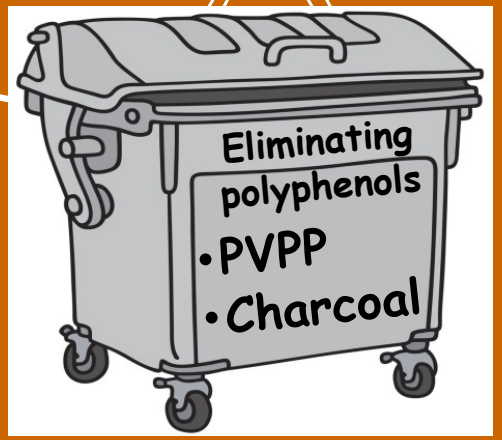


Secondary quinone

Repeated Several folds

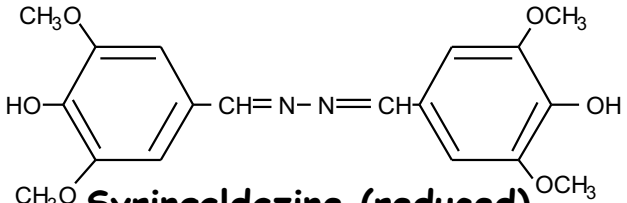
Condensation

Other phenols



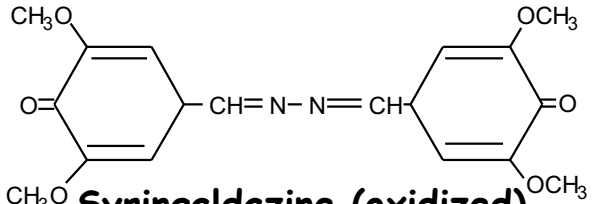
Measurement of Laccase activity

Vignault et al., (2019) *Oeno One*, **1**, 27-38



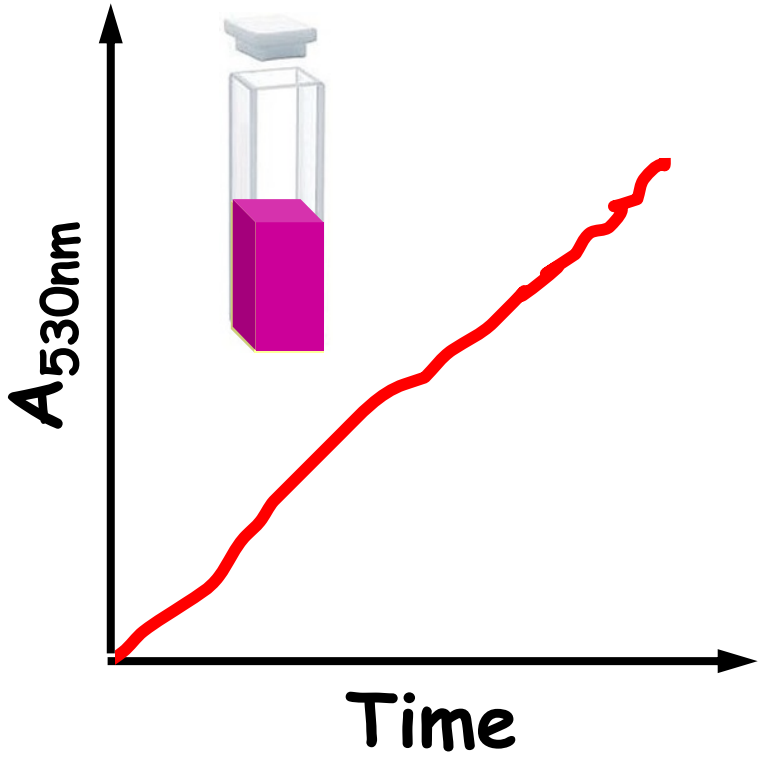
Syringaldazine (reduced)
COLORLESS

O_2 → **Laccase**



Syringaldazine (oxidized)
PINK-RED

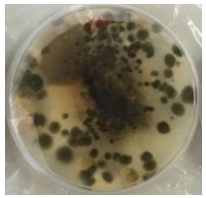
Absorbance measurement at 530 nm



Experimental design

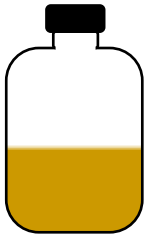
Vignault et al., (2019) *Oeno One*, **1**, 27-38

Reproduction of *B. cinerea* 213 on YPD plates



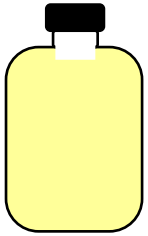
2 weeks;
20 °C
90-100%
of humidity

Botrytized must



Inoculation by spraying spore suspension

Healthy grapes



Healthy Grape juice

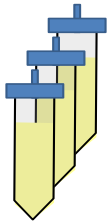
17 mL

22 mL

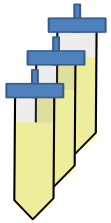
5 mL

Laccase Inhibition Assays

Direct measurement of Laccase activity with or without addition of tannins



1.5 UL/mL

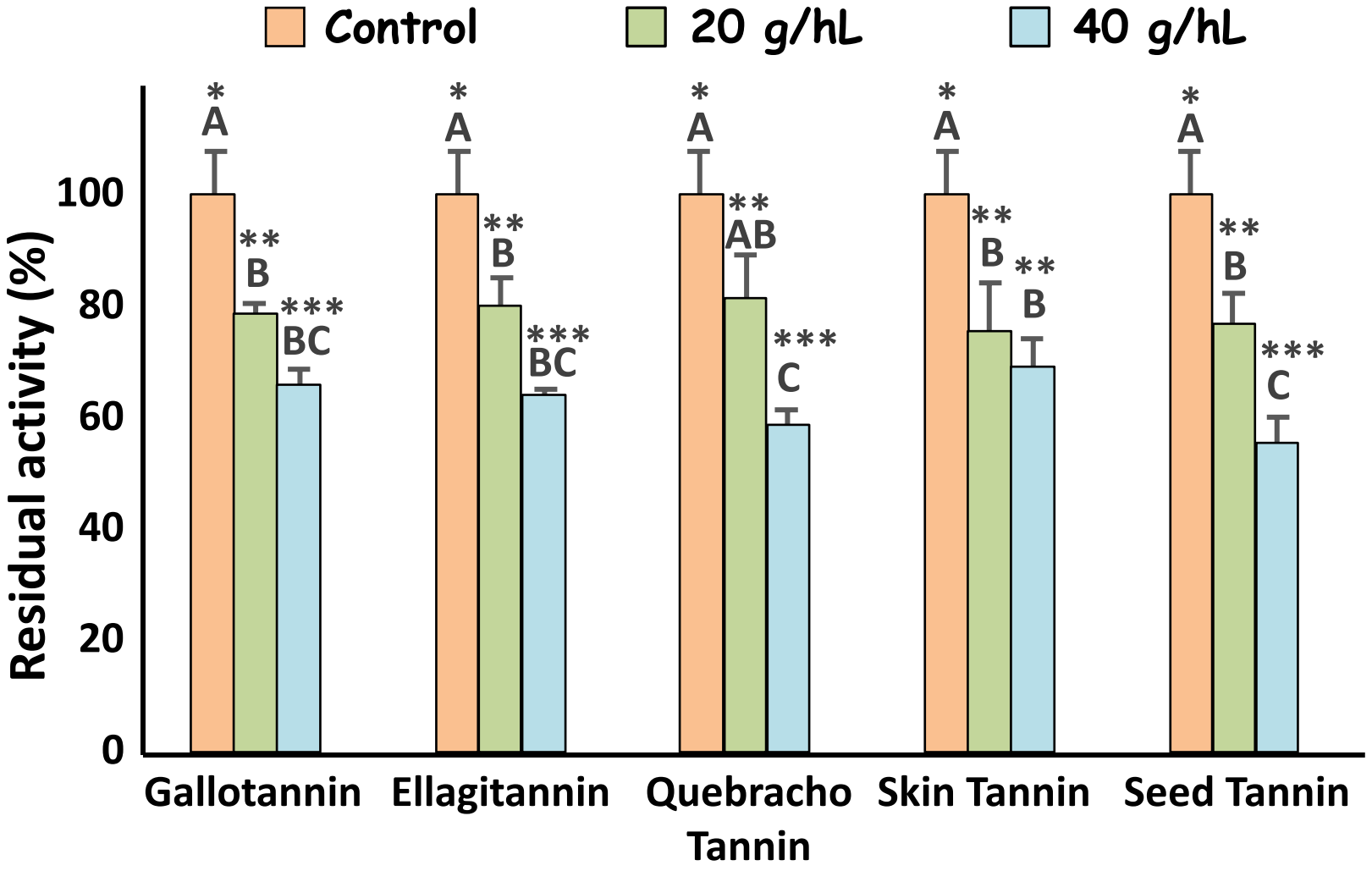


0.0 UL/mL

Alcoholic fermentation with or without addition of tannins
Color measurements of wines

Impact of oenological tannins on laccase activity

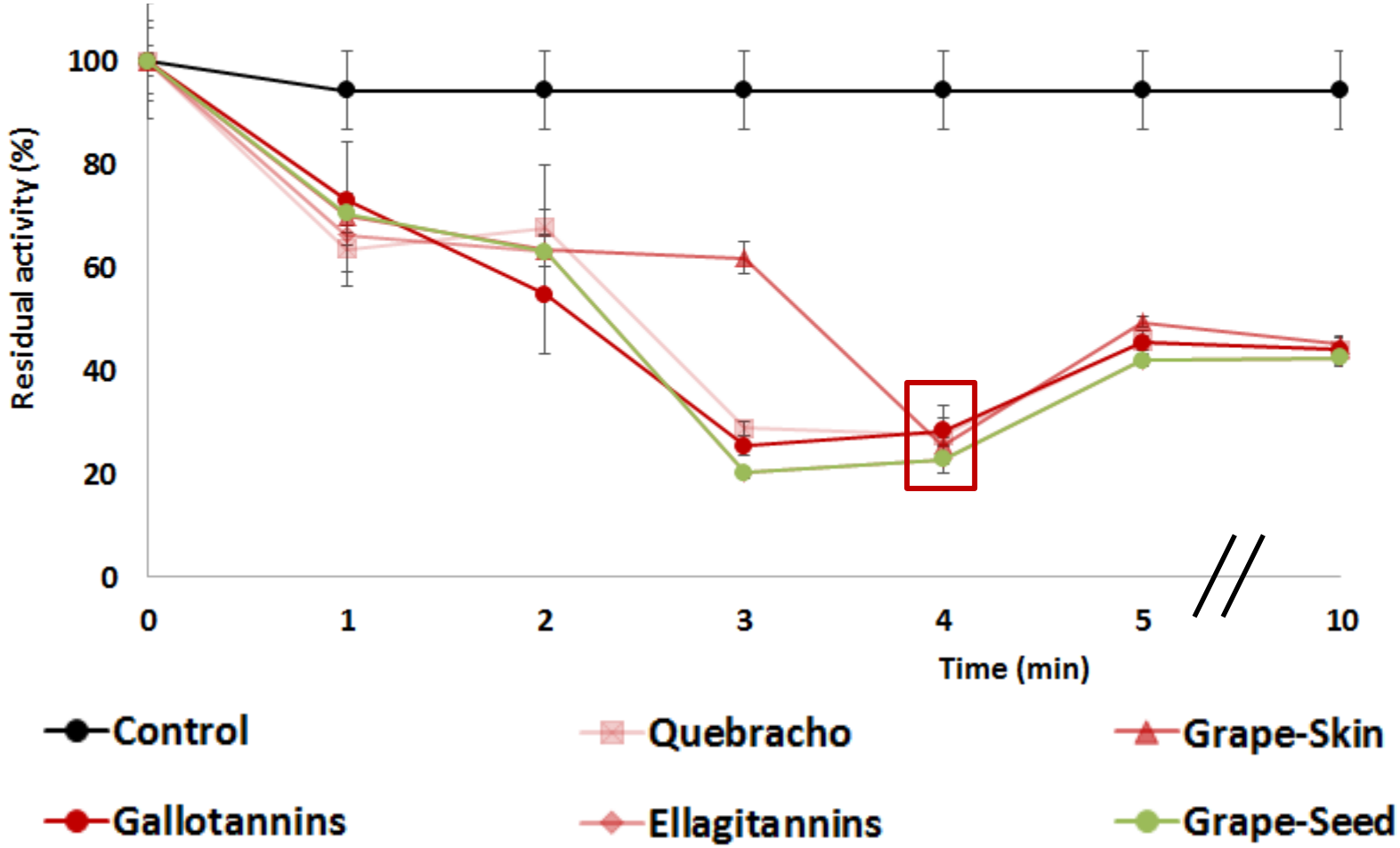
Vignault et al., (2019) *Oeno One*, **1**, 27-38



Influence of contact time on laccase activity

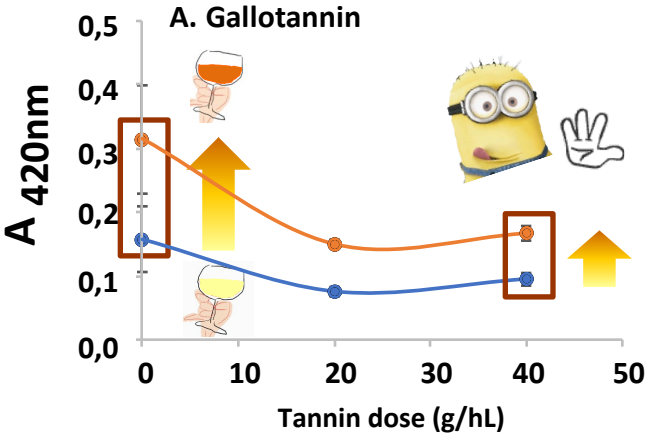
Vignault et al., (2019) *Oeno One*, **1**, 27-38

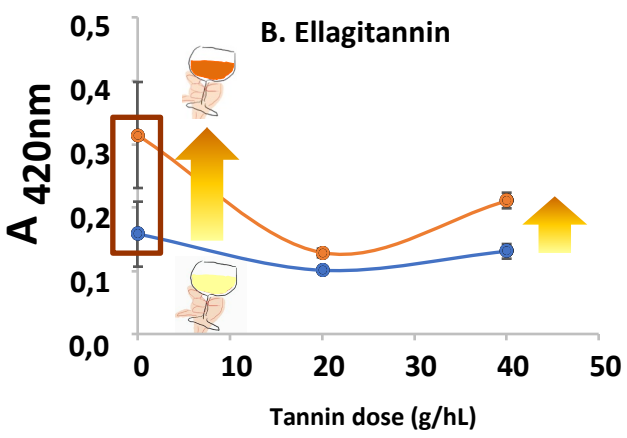
Time contact needed : 4 minutes



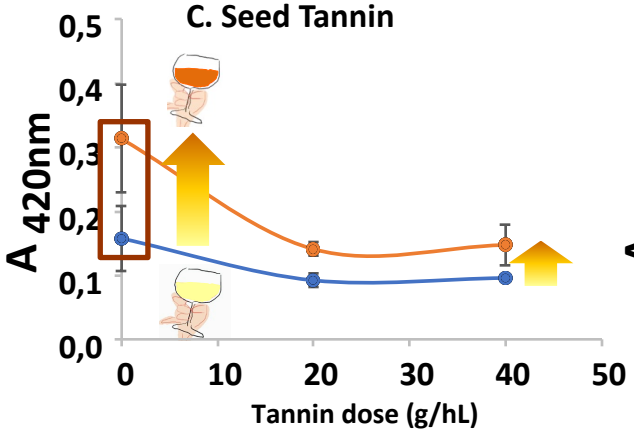
Antilaccase capacity; white winemaking

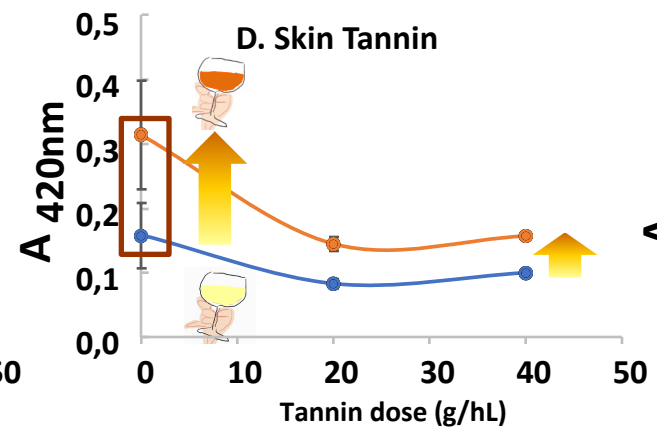
Vignault et al., (2019) *Oeno One*, **1**, 27-38

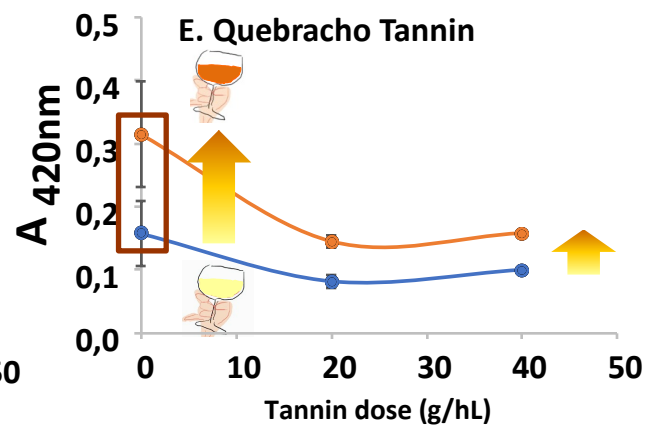




Without laccase With laccase

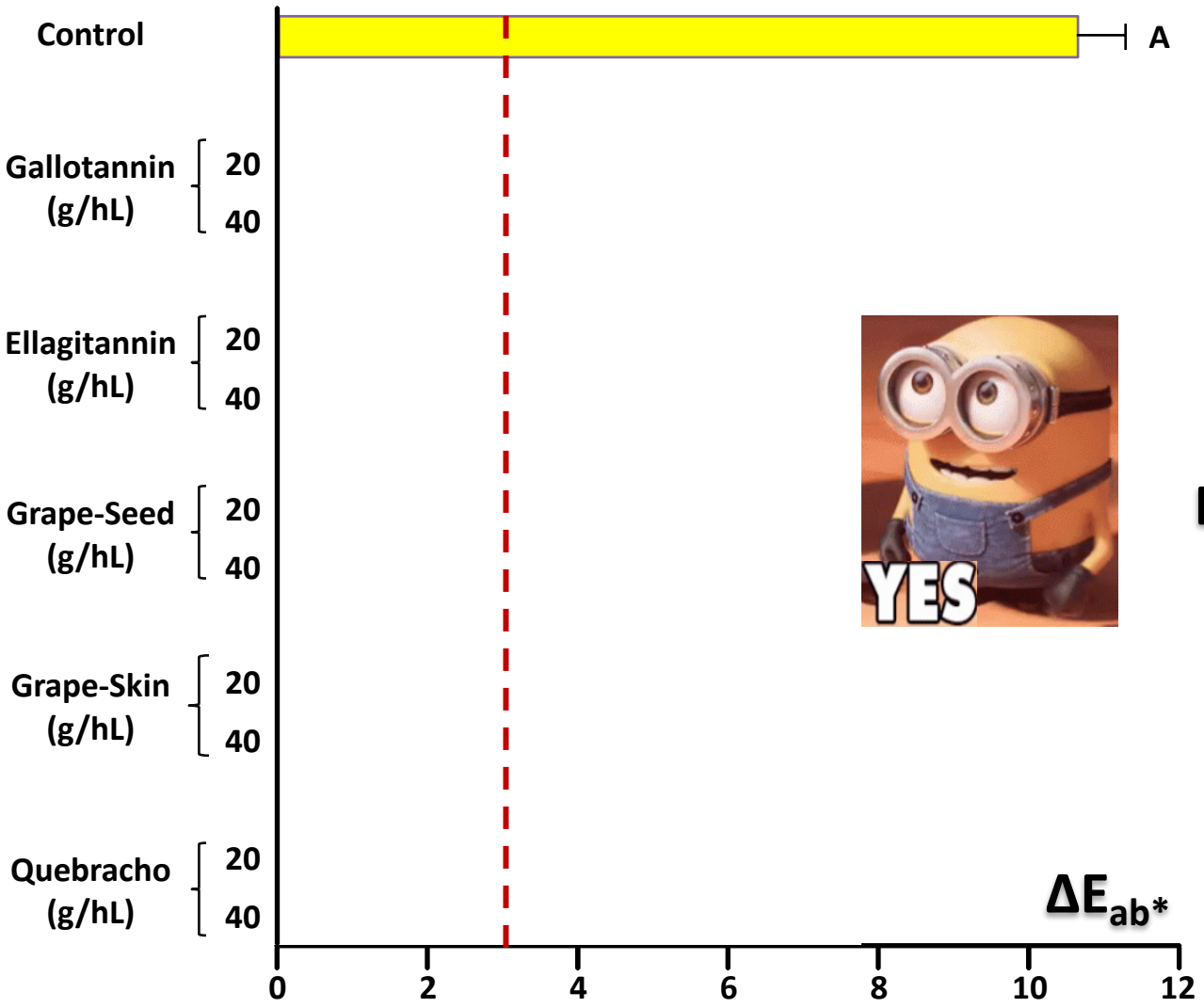




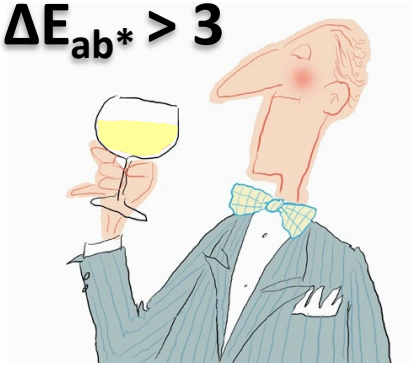


Antilaccase capacity; white winemaking

Vignault et al., (2019) *Oeno One*, **1**, 27-38



$\Delta E_{ab^*} > 3$



The human eye can distinguish

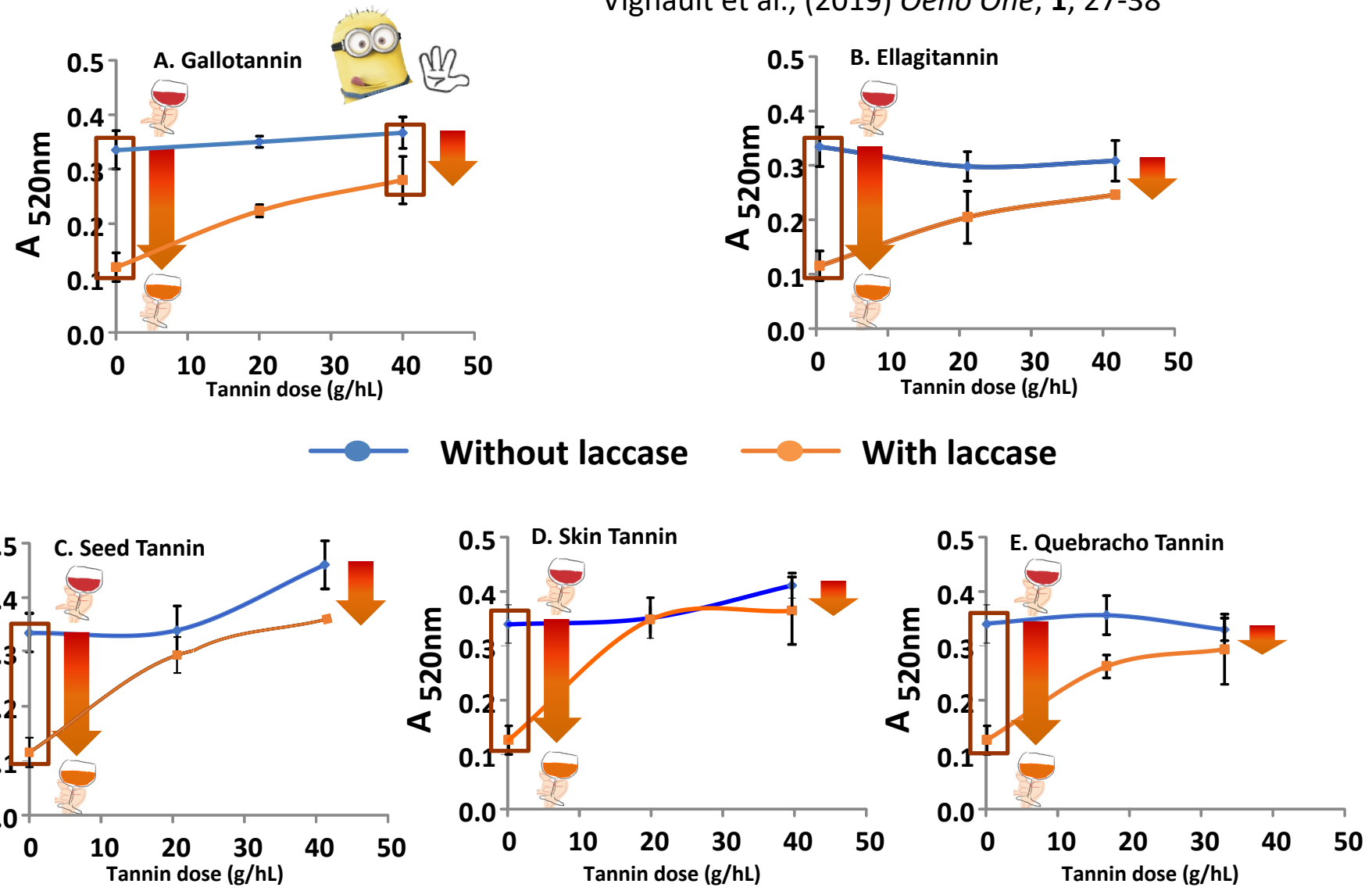
But when

$\Delta E_{ab^*} < 3$

The human eye cannot distinguish

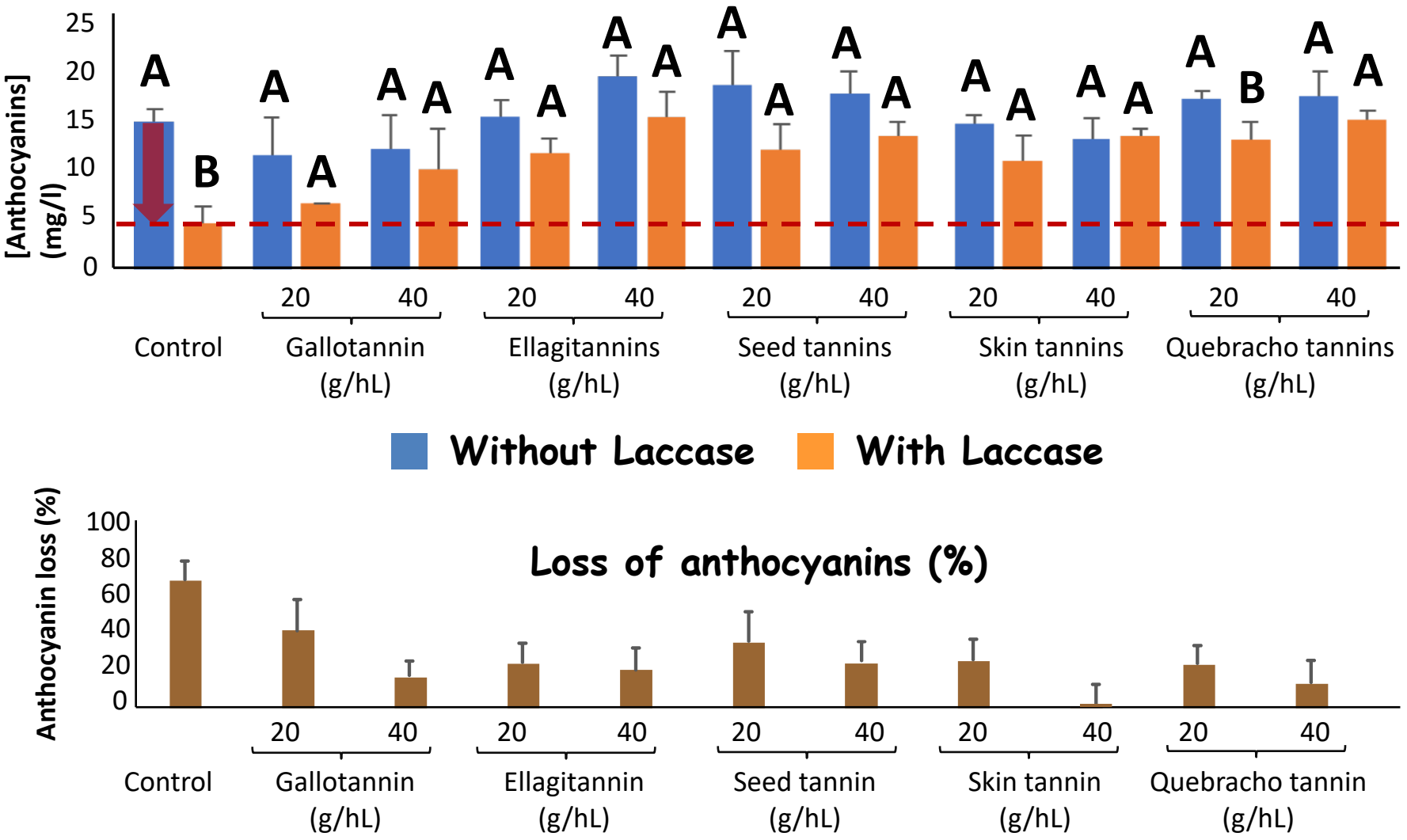
Anti-Laccase capacity; red winemaking

Vignault et al., (2019) *Oeno One*, **1**, 27-38



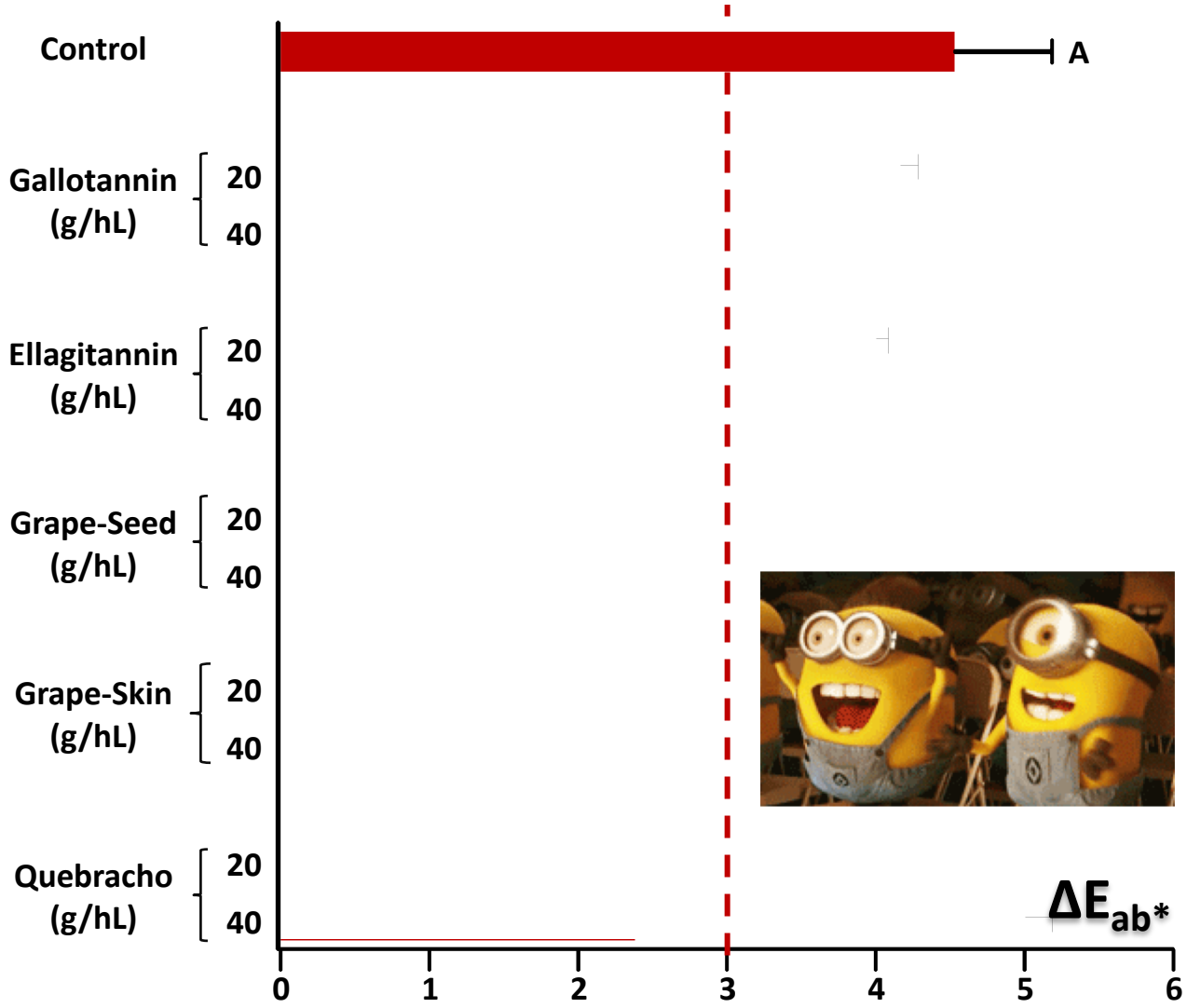
Anti-Laccase capacity; red winemaking

Vignault et al., (2019) *Oeno One*, **1**, 27-38

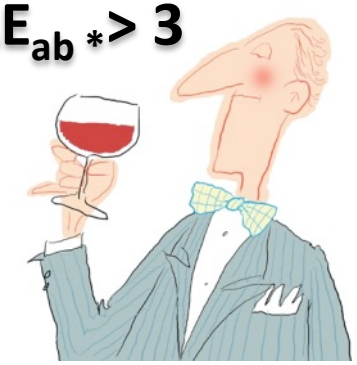


Anti-Laccase capacity; red winemaking

Vignault et al., (2019) *Oeno One*, **1**, 27-38



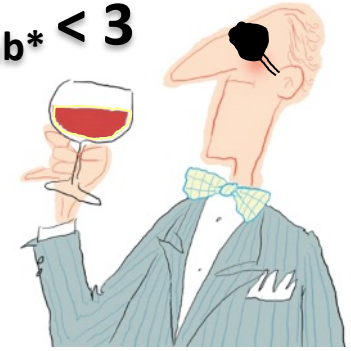
$\Delta E_{ab}* > 3$



The human eye can distinguish

But when

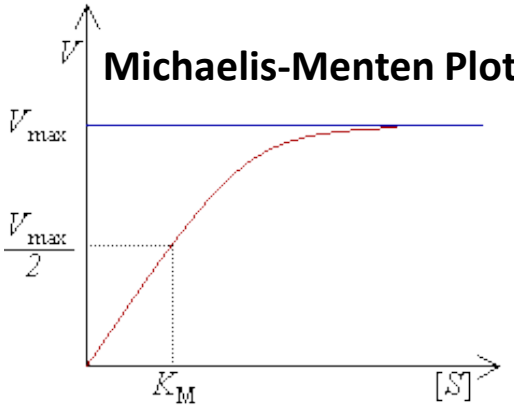
$\Delta E_{ab}* < 3$



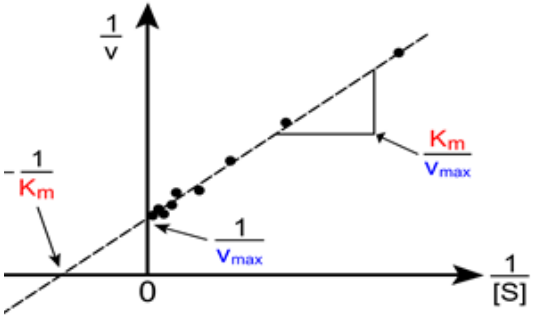
The human eye cannot distinguish

Comprehension of action mechanisms of oenological tannins on laccases produced by *B. cinerea*

Michaelis-Menten Plot

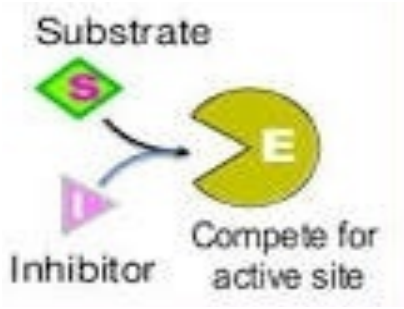


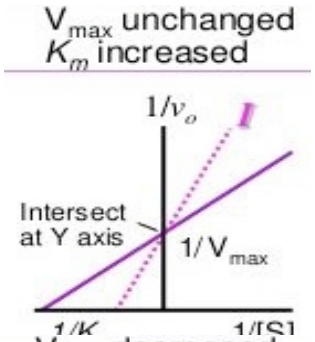
Lineweaver-Burk Plot



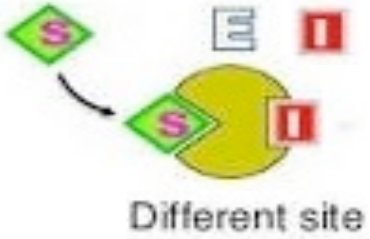
Type of inhibition

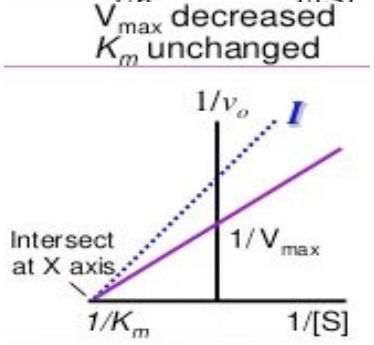
Competitive



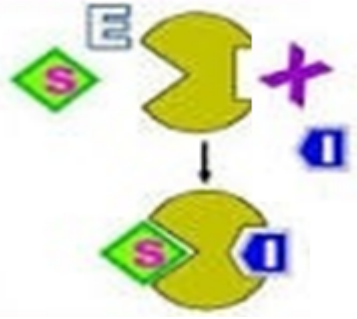


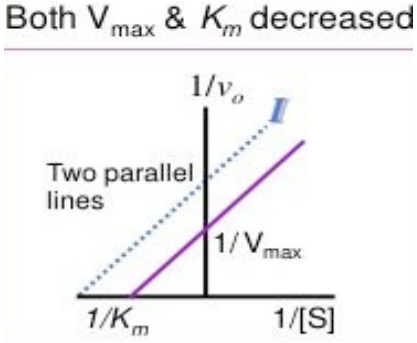
Non-competitive





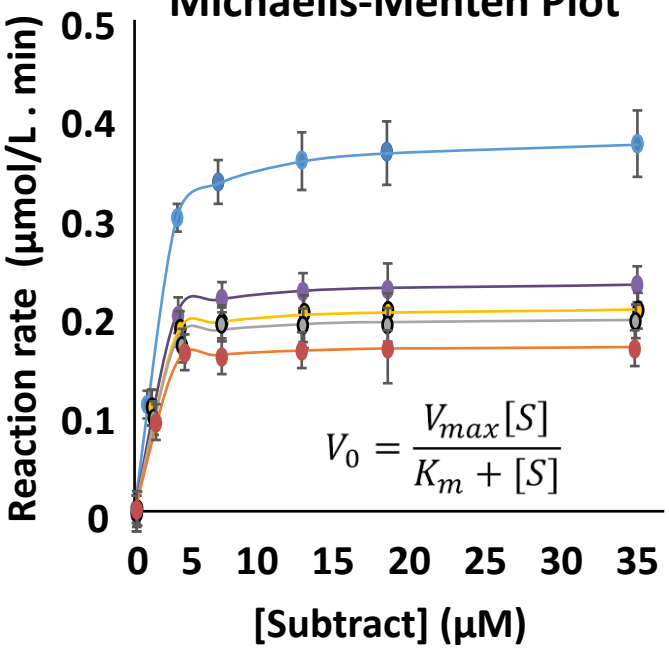
Uncompetitive





K_M = Affinity of the enzyme for the substrate
V_{max} = Maximal reaction rate

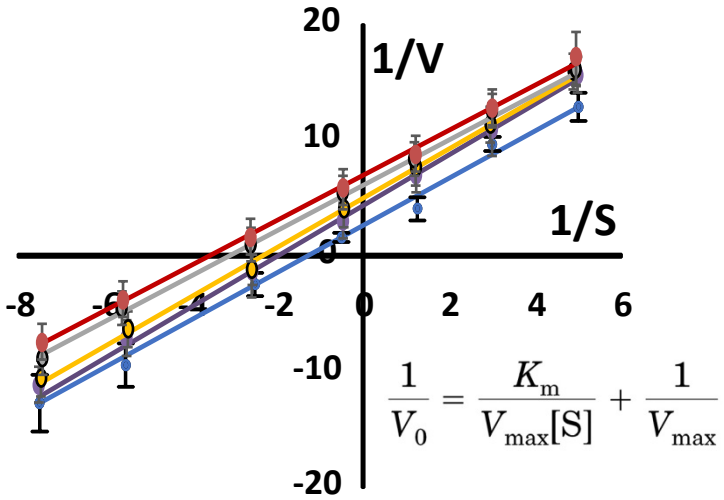
Michaelis-Menten Plot



Grape-Seed tannins

- 0 g/hL
- 10 g/hL
- 20 g/hL
- 30 g/hL
- 40 g/hL

Lineweaver-Burk Plot



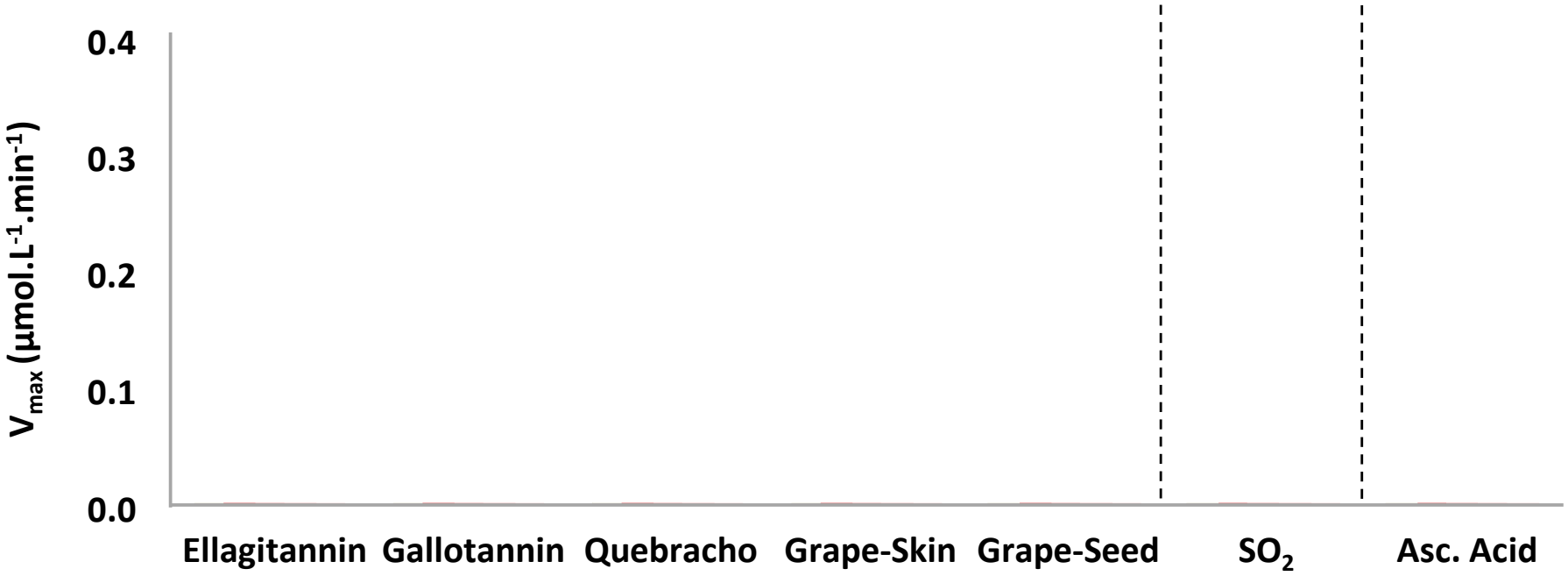
Uncompetitive inhibition

Oenological tannins : V_{max} and K_M diminished compared to the control → **Uncompetitive inhibitors**

Sulfur dioxide : V_{max} and K_M diminished compared to the control → **Uncompetitive inhibitor**

Ascorbic acid : V_{max} diminished and K_M remain constant compared to the control → **Non competitive inhibitor**

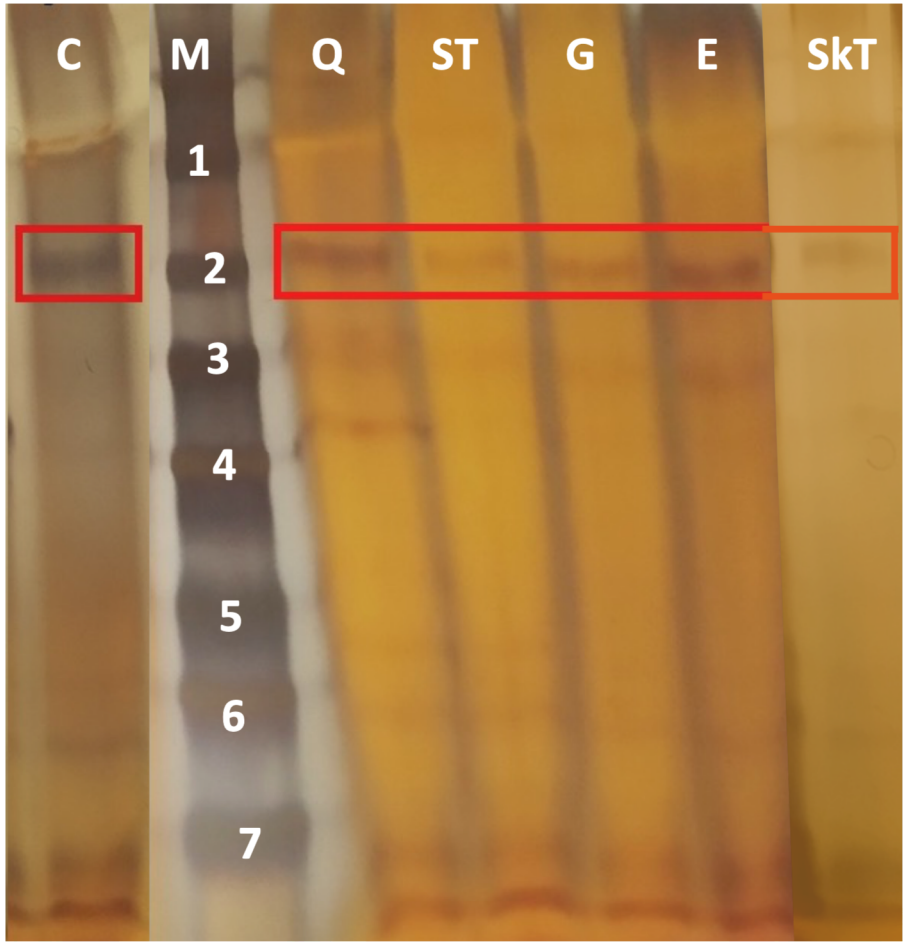
Comparison of the Laccase inhibitory effectiveness



PAGE-SDS Electrophoresis

M: Molecular Weight marker

1: 140 kDa
2: 95 kDa
3: 52 kDa
4: 34 kDa
5: 26 kDa
6: 17 kDa
7: 10 kDa



	Sample	Band intensity
C	Control	100%
Q	Quebracho Tannin	105%
ST	Seed Tannin	23%
G	Gallotanin	48%
E	Ellagitannin	94%
SkT	Skin Tannin	26%

Conclusions

Oenological tannins are an interesting tool for protecting grape juice against laccase browning which can help to reduce SO_2 doses

Thank you very much for your attention

The authors are grateful to to Oenoppia, Laffort industries and to Spanish Ministerio de Agricultura (CICYT:Projects AGL2014-56594-C2) for their financial support. We are also grateful to OIV for the scholarship granted to Jordi Gombau and to the French Ministry of Research and Technology for the scholarship granted to Adeline Vignault (CIFRE ANRT grant number 2016-0823).