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01
Clarification of the must : the role of Enzymes

02
Natural tools to protect your wines from Oxidation

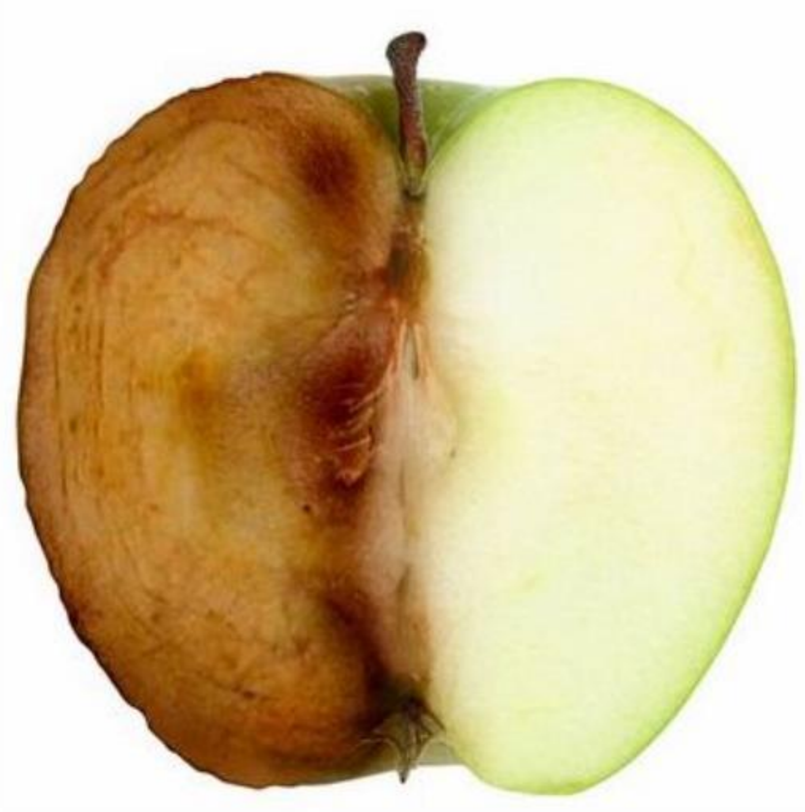
03
How to produce and preserve Aromas

IOC

1. Origin and mechanisms of must oxidation

What is must oxidation in oenology?

- **<u>Irreversible</u>** reaction
- Ageing phenomenon linked to an **<u>enzymatic</u>** reaction
- The result is a **<u>loss of quality</u>** :
  - Must browning
  - Degradation of thiol aromas
  - Increased dryness and bitterness



<u>Almost exclusively enzymatic in origin</u>
If the musts are heated to destroy the enzymes, the rate of consumption falls from 2.95 to 0.018 mg O_2/L/min.

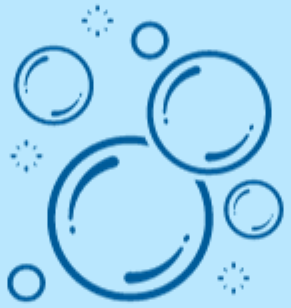
Be careful, <u>the aromatic loss</u> may occur before the colour change.

Two phenomena: dissolution and consumption

1 Dissolution

The oxygen passes from the gaseous phase to the liquid phase.

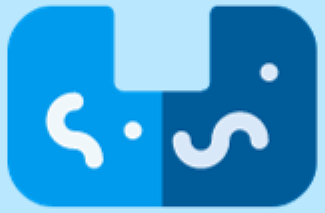
Oxygen levels that can be dissolved: 8.4 mg/L at 20°C.



2 Consumption

Enzymatic consumption of oxygen contained in the must.

Doses of oxygen that can be consumed: 80 mg/L for whites, 800 mg/L for reds.



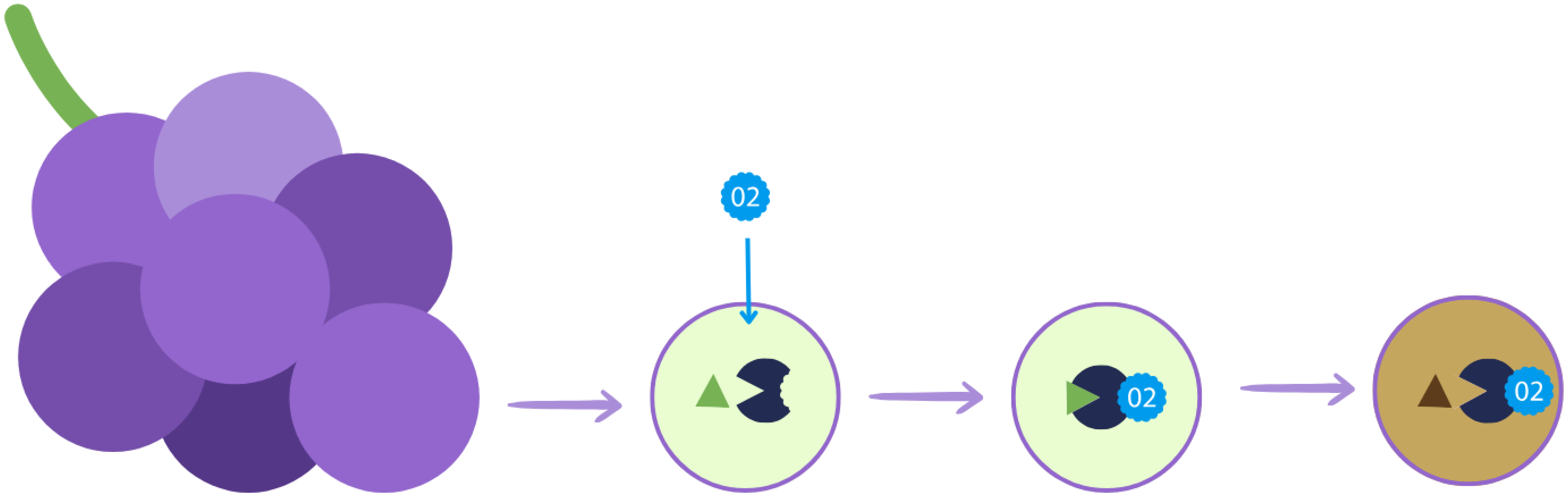
Oxygen is consumed as it dissolves, up to 10 times saturation.

Oxygen consumption

- To oxidise musts, you need :
  - Molecular dissolved oxygen 02
  - Oxidase enzymes
  - Oxidation substrates

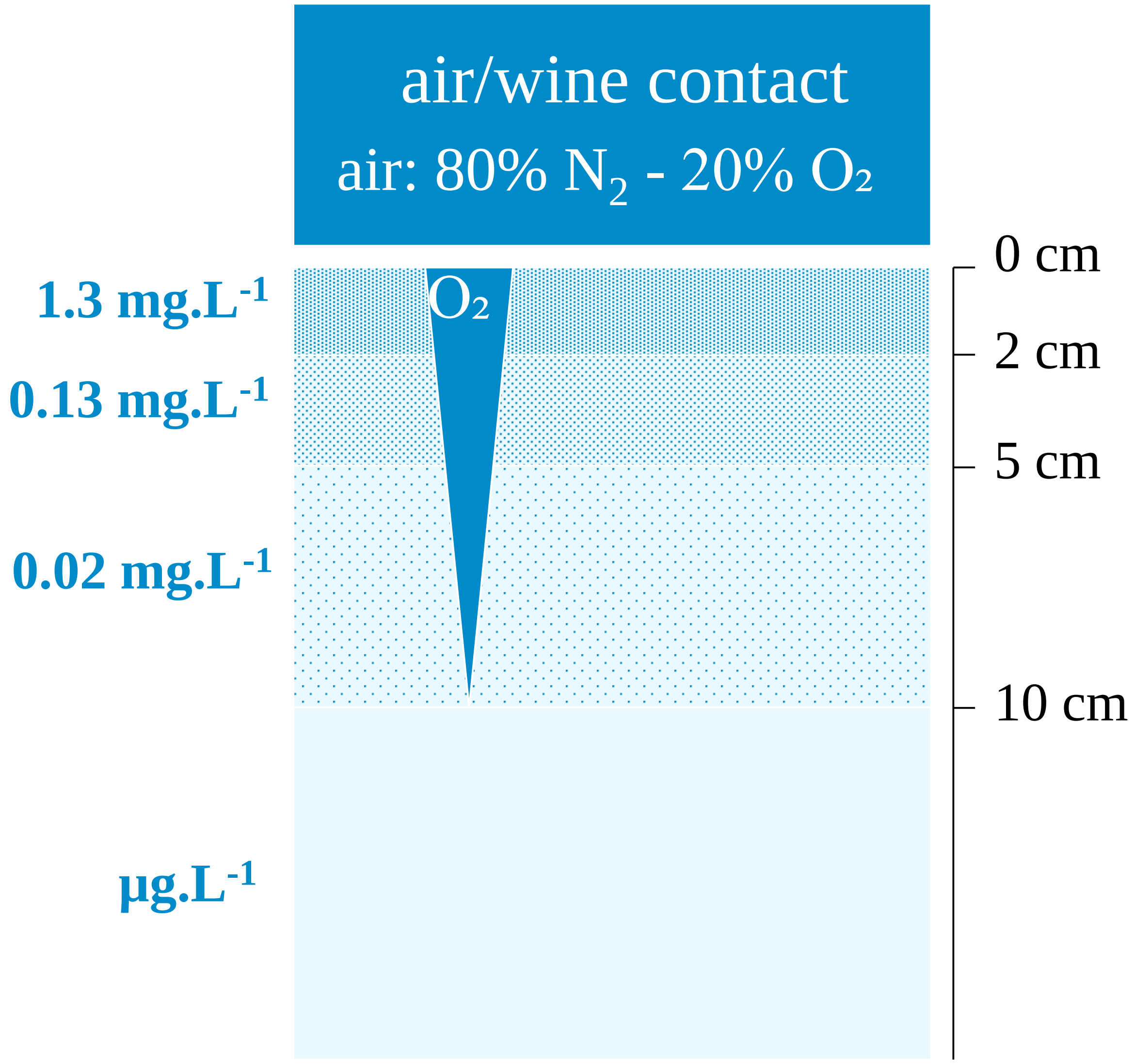
QUINONES → Browning

QUINONES → Loss of aromas



Dissolving oxygen 02

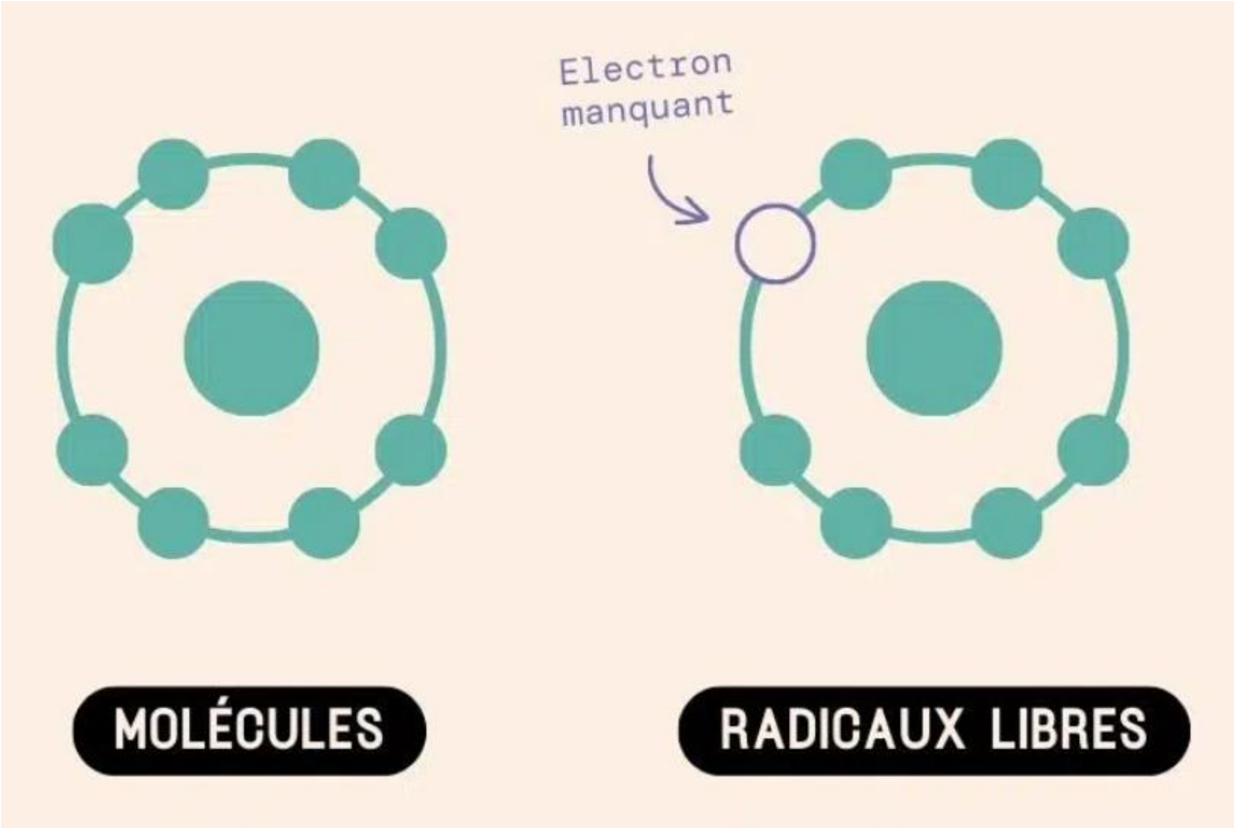
- Occurs on **contact** between the air and the liquid:
  - At harvest time
  - At the crushing stage
  - At the time of pressing
  - Filling the settling tank
  - ...
- Follows **Henry's law**: $PO_2 = H.C^*$.
  - $PO_2 = 0.2$ (partial pressure of O_2 in the gas)
  - $C^* = 8.4\ mg.L^{-1}$ (maximum oxygen solubility)
- The **lower** the **temperature**, the faster the oxygen dissolves

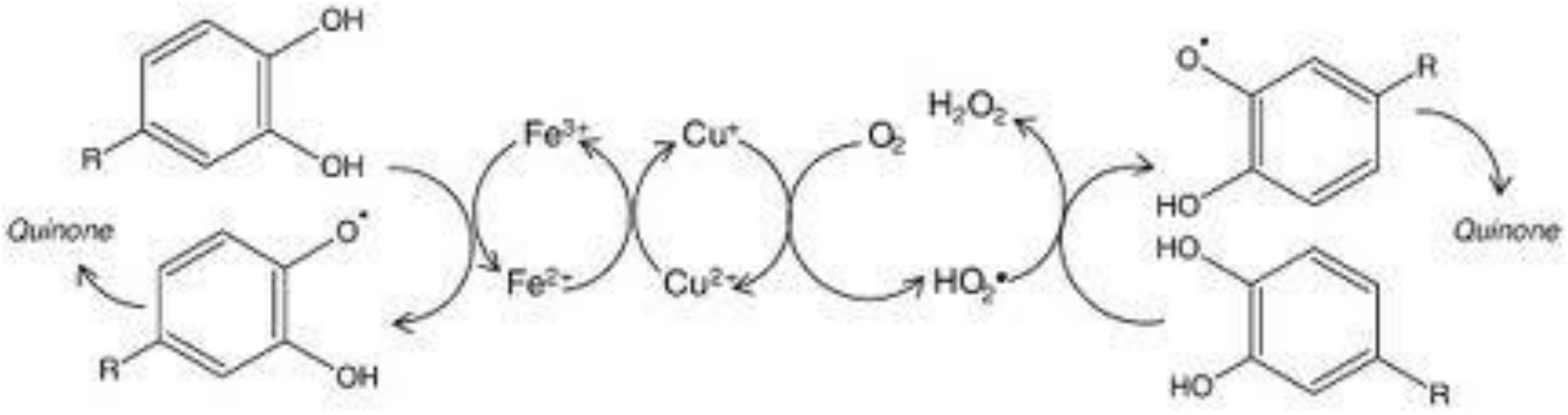


(Moutounet and Mazariuc, 2001)

Formation of free radicals

- Oxygen changes to **the molecular state** (free radical), losing an electron
- This reaction is catalysed by **Cu** and **Fe**





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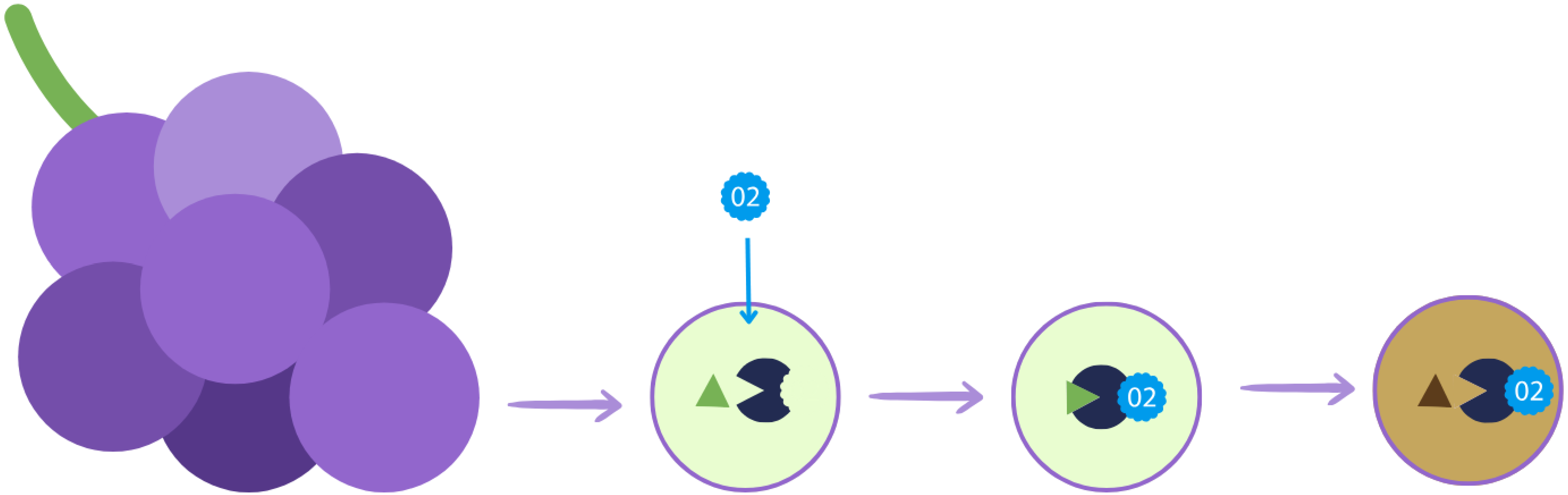
  - Oxidation substrates

QUINONES

Browning

Loss of aromas



Oxidases

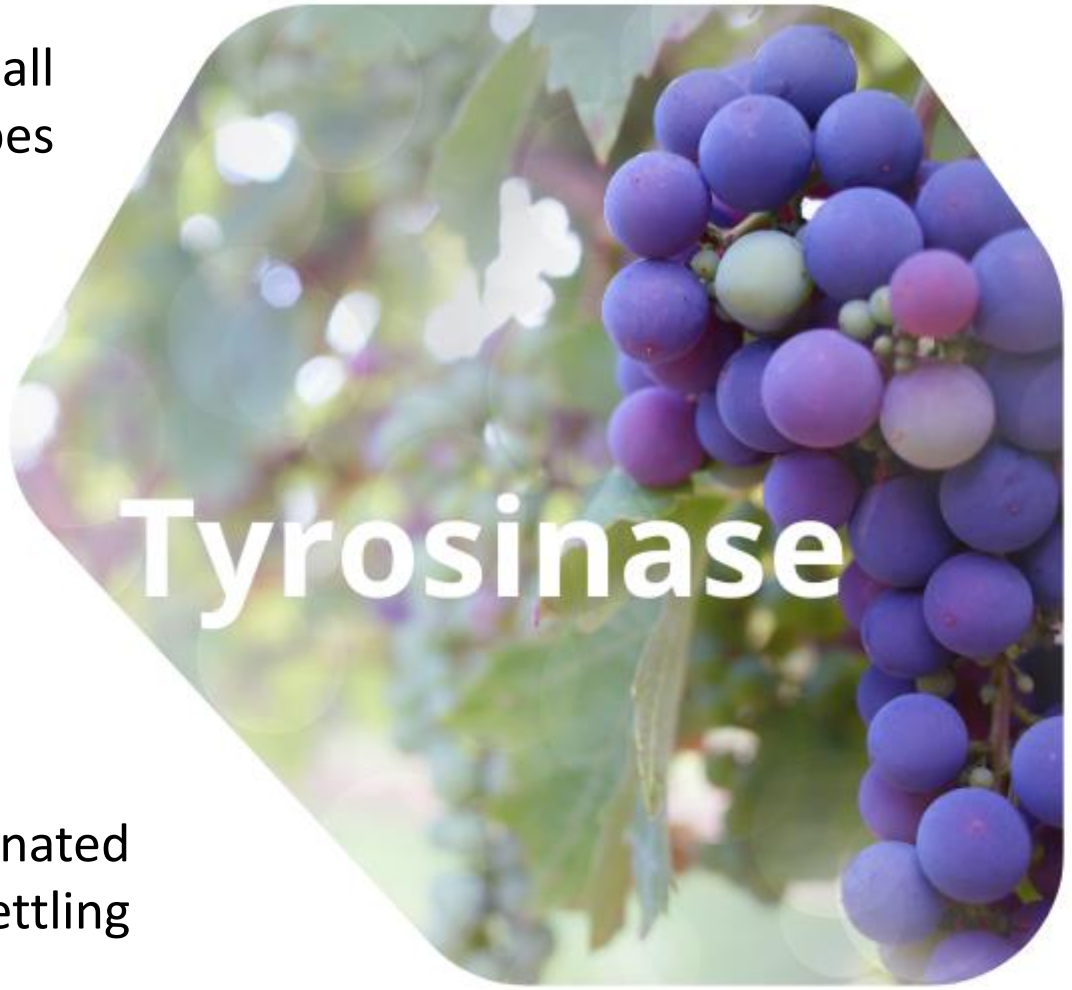
Polyphenol oxidases (PPO): catalysts for the oxidation of phenolic compounds in the presence of molecular oxygen

Present on all healthy grapes

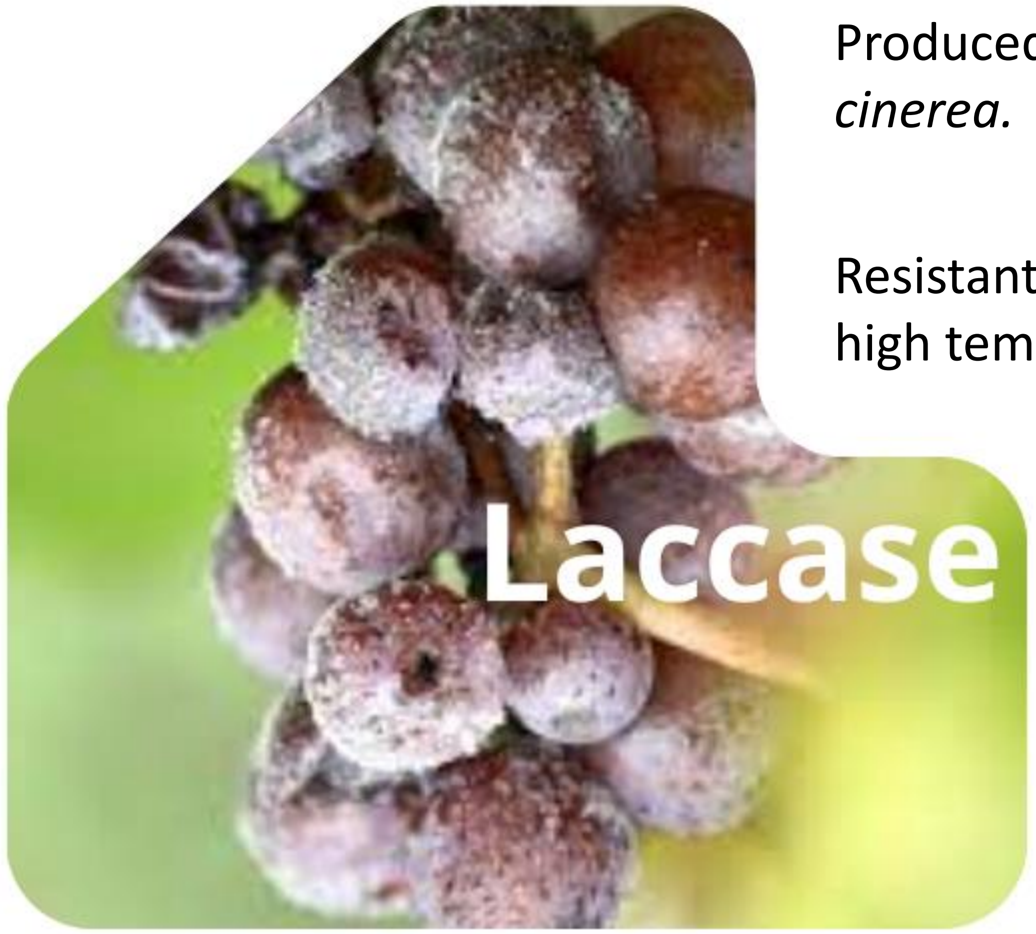
Not very stable

Sensitive to tannins and SO_2

Partly eliminated during settling



Rapid oxidation of hydroxycinnamic acids



Produced by *Botrytis cinerea.*

Resistant to SO_2 , acidic pH and high temperatures

Very stable and soluble, remains after settling

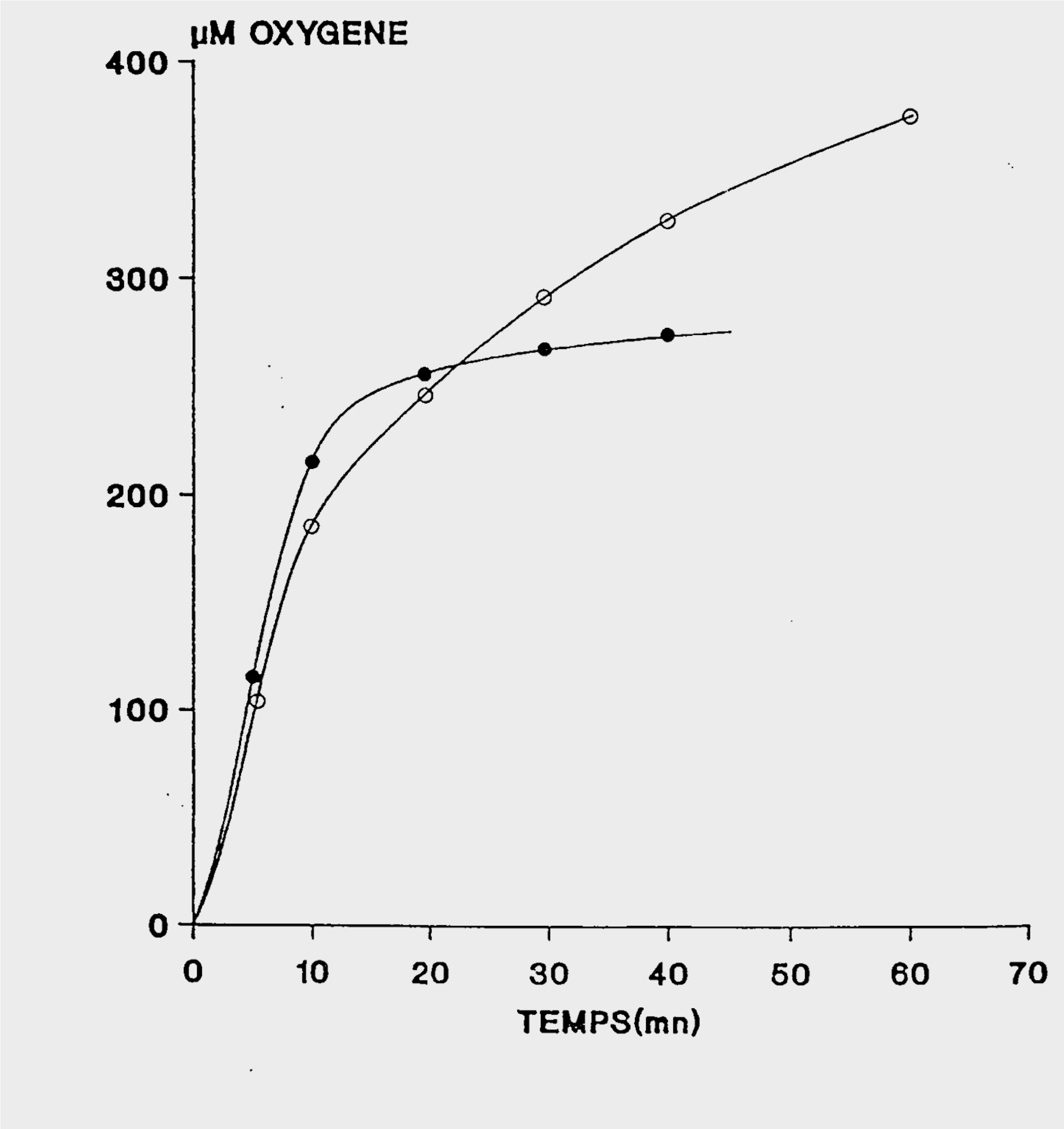
Inhibited by gallic tannins

Oxidises all phenolic compounds **very quickly**

Kinetics of oxygen consumption by oxidases

- In healthy must :
  - Oxygen consumption kinetics are rapid at first, then decrease significantly
- In botrytised must :
  - The capacity to consume oxygen is much greater

Kinetics of oxygen consumption in colombard must



Control must with healthy berries

Must from a sample with 10% parasitized berries

Sensitivity to SO_2

- In healthy must :
  - An addition of 5 g/hL of SO_2 results in a 90% loss of oxidase activity after 5 minutes of contact.

SO_2 (mg/L)	Loss of oxidase activity (%)
0	0
5	24
50	90
100	99,5

- In botrytised must :
  - At 15 g/hL SO_2 , only 20% of oxidase activity is inhibited.

SO_2 (mg/L)	Loss of oxidase activity (%)
50	10
150	20

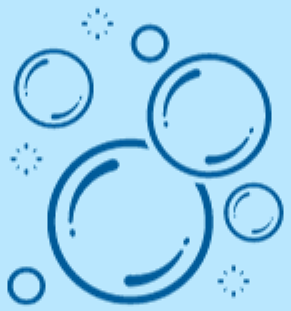
The role of temperature: a real time bomb

1

Dissolution

Faster oxygen dissolution at **<u>lower temperatures</u>**.

At 5-8°C, oxygen accumulates rapidly in the must.

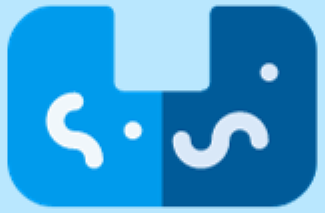


2

Consumption

Faster oxygen consumption at **<u>high temperatures</u>**.

At 5-8°C, the enzymes are only slightly active and little oxygen is consumed.



The dissolved oxygen reserve is ready to be consumed as soon as the temperature rises!

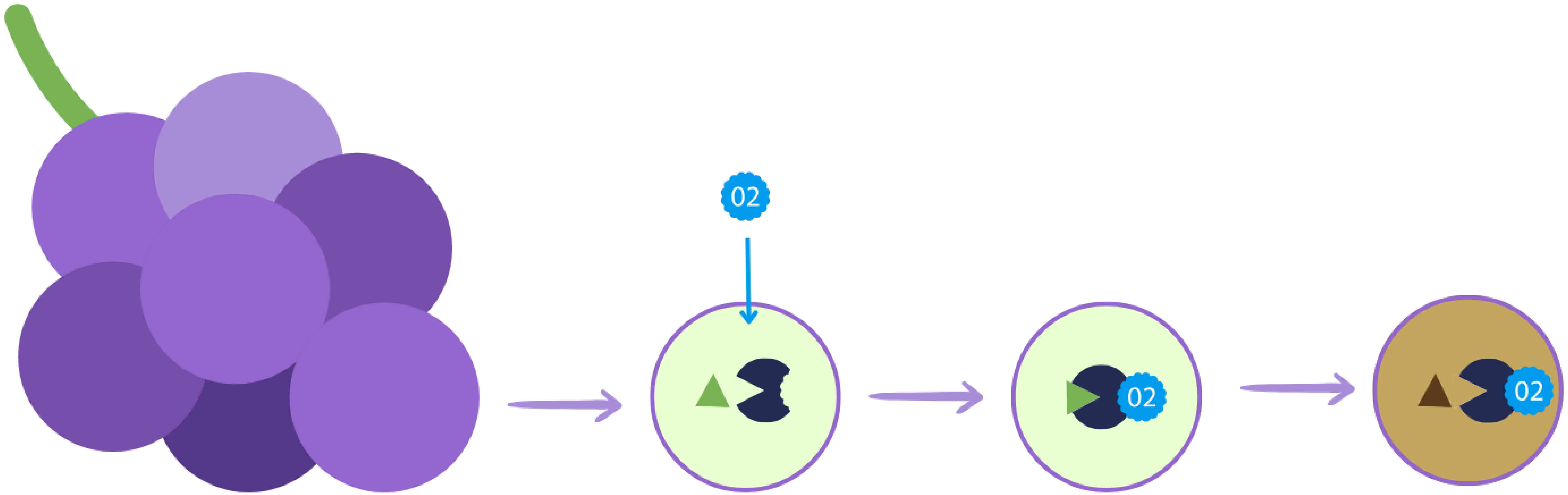
Oxygen consumption

- To oxidise musts, you need :
  - Molecular dissolved oxygen 02
  - Oxidase enzymes
  - Oxidation substrates

QUINONES

Browning

Loss of aromas

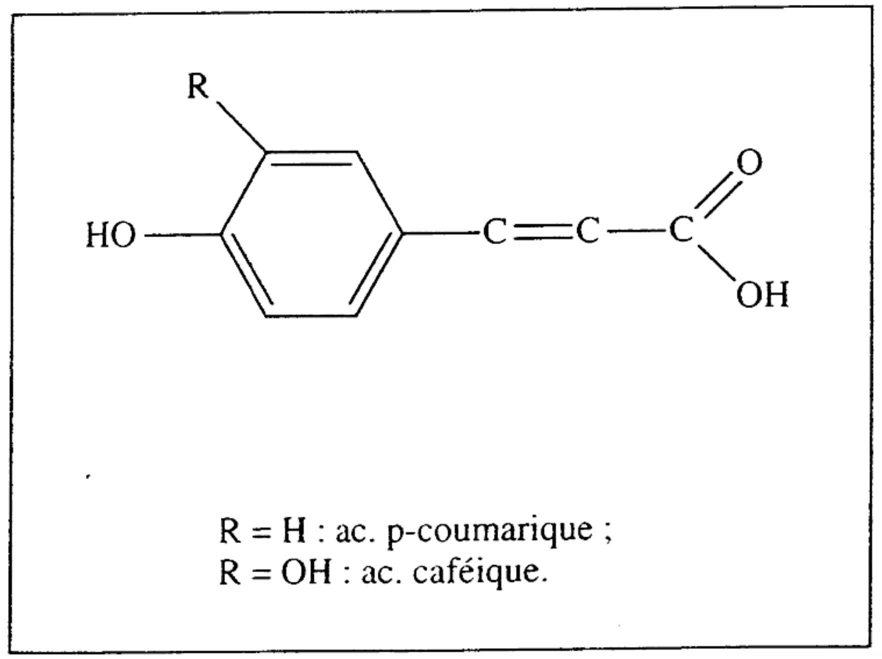


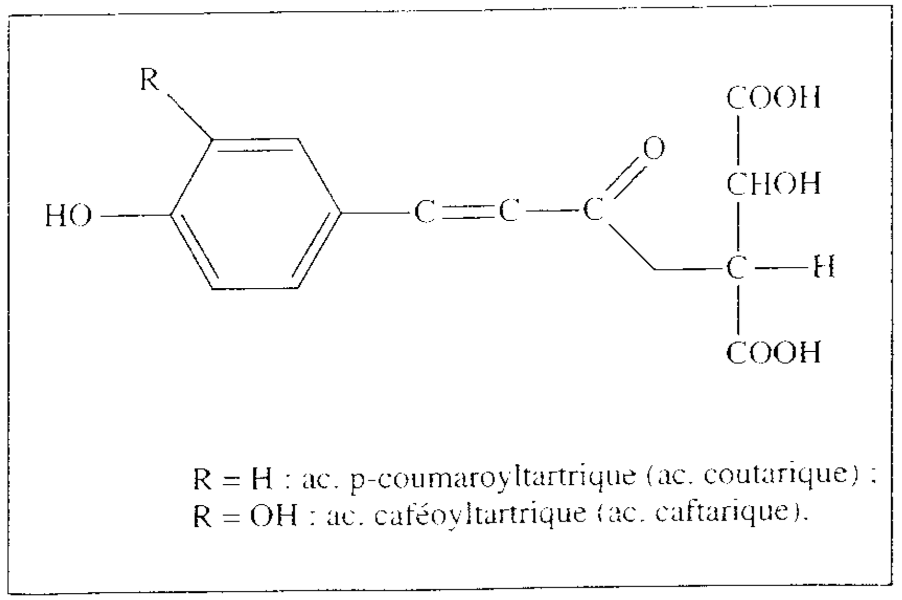
Hydroxycinnamic acids

- Hydroxycinnamic acids (**<u>HA)</u>** in grapes :
  - Coumaric acid
  - Caffeic acid
  - Coutaric acid
  - Caftaric acid

- Phenol acid family

- **<u>Tyrosinase</u>** and **<u>Laccase</u>** substrates





O_2 consumption capacity according to grape variety

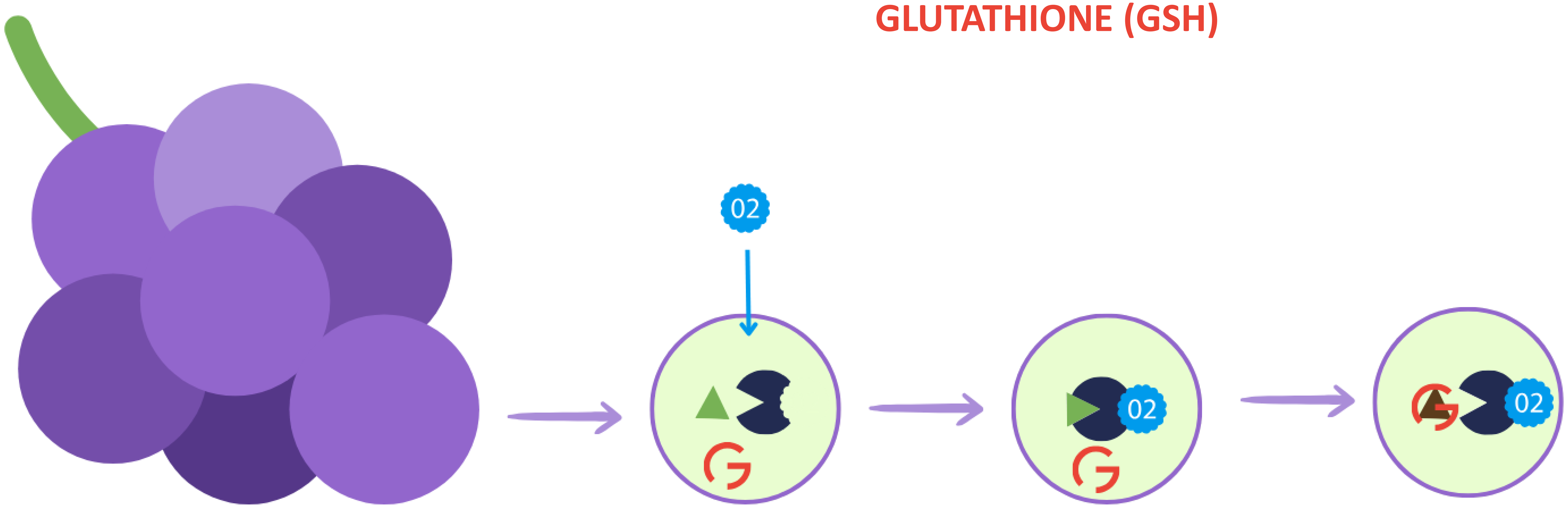
- HA content of the grape variety: helps to determine its oxidation potential

Grape varieties	Consumption of O_2	HA content
Chasselas	180	152
Chenin	240	208
Colombard	270	254
Pinot noir	360	239
Pinot blanc	460	316
Sauvignon	520	428
Grenache	610	480
Muscat de frontignan	810	715
Chazan	940	853

Estimated oxygen consumption capacity (µmoles/l); AH = Hydroxycinnamic acids in musts obtained in the absence of air.

The protective effect of Glutathione

- To oxidise musts, you need :
  - Molecular dissolved oxygen
  - Oxidase enzymes
  - Oxidation substrates



The grape variety's glutathione content determines its capacity to consume O_2

- The **HA/GSH ratio** is a good indicator of the capacity of musts to brown.
- This ratio is related to :
  - Grape variety
  - Climatic conditions
  - Must extraction conditions
- The HA/GSH ratio can be used to determine the oxygen strategy:
  - [AH] < [GSH], favour a reductive approach
  - [AH] > [GSH], possible use of oxygen

[HA]/[GSH] <1	[HA]/[GSH]1-3	[HA]/[GSH]>3
Colombard Chenin Chasselas Clairette	Chardonnay Sauvignon blanc Mauzac Pinot Blanc Ugni Blanc	Gros Manseng Burgundy melon Riesling

Cheynier et al.

Hyperoxygenation of wines

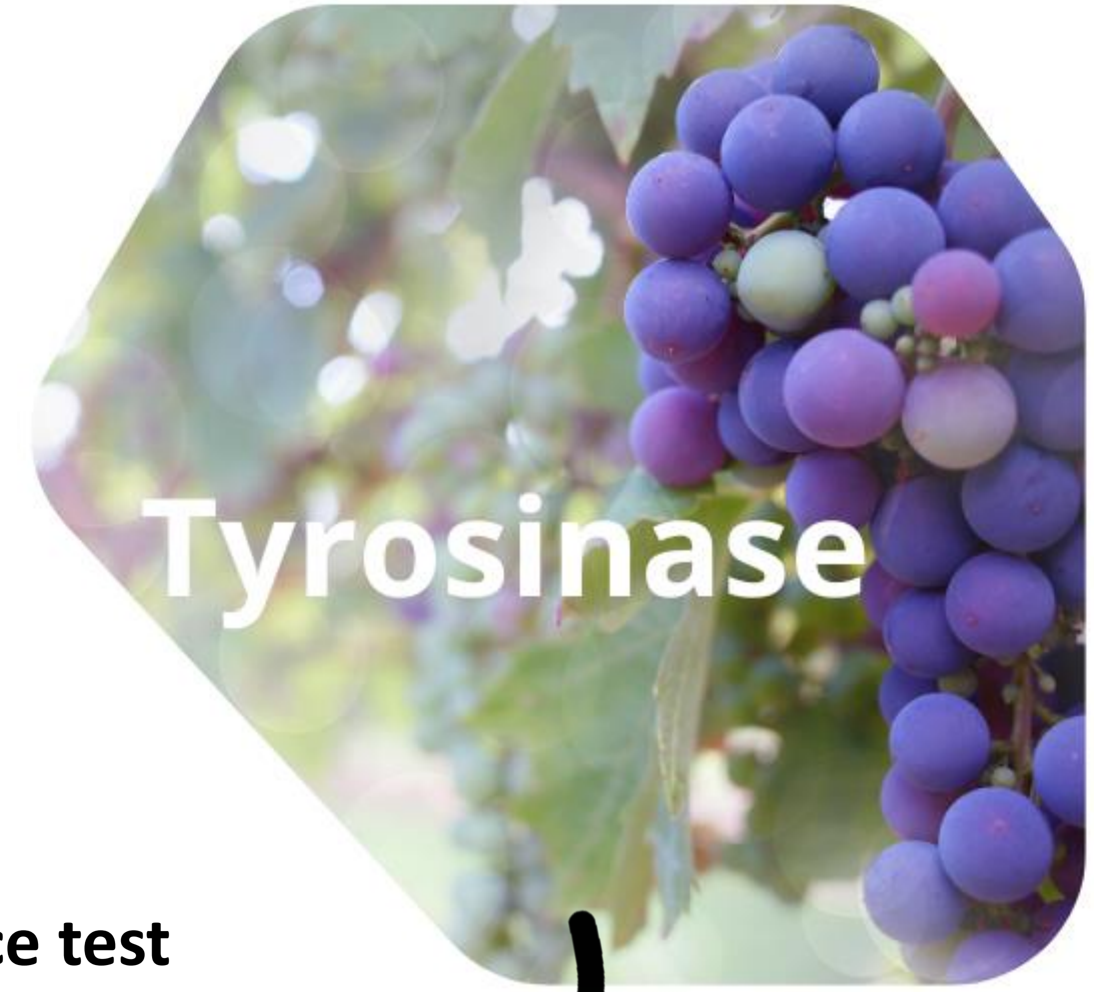
- Principle :
  - Injecting pure oxygen into the heart of the must
  - Forced and massive oxidation of phenolic compounds
  - Condensation and precipitation > intense browning
  - Elimination of these substances during settling

- IOC recommendations :
  - Mixed results
  - Rapid oxidation and loss of glutathione
  - Loss of flavour and impact on quality
  - Prohibited in the presence of laccase

  > Can be used in the case of **very spoilt grapes**

How to assess the oxidative capacity of musts

Laccase test



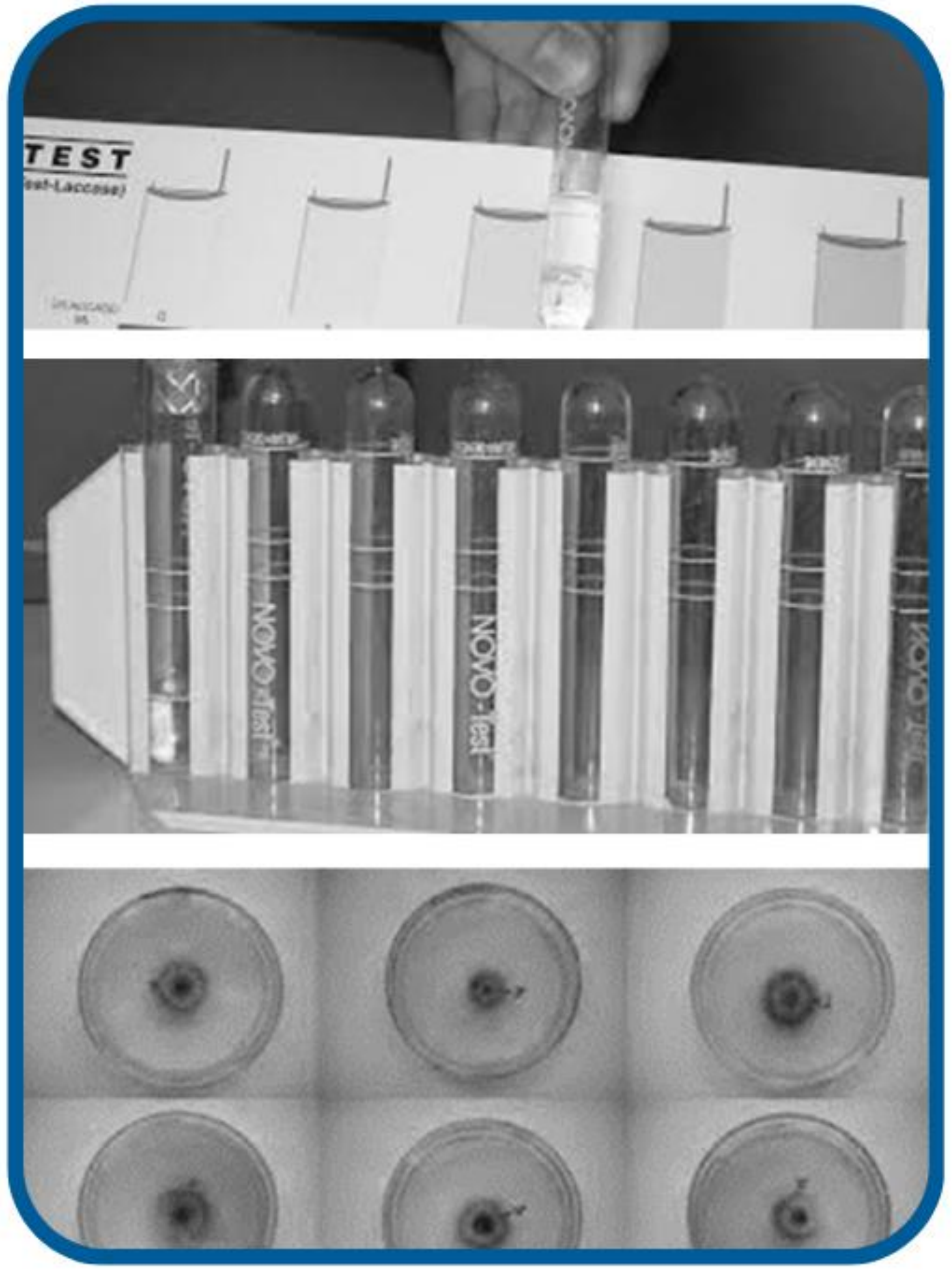
Air resistance test

Air resistance test

- Place the wine in contact with the air for 2-3 hours.
- Visual assessment of colour evolution
- Tasting before and after contact with air

Laccase test

- Colorimetric test (kit)
- Several tests are available on the market

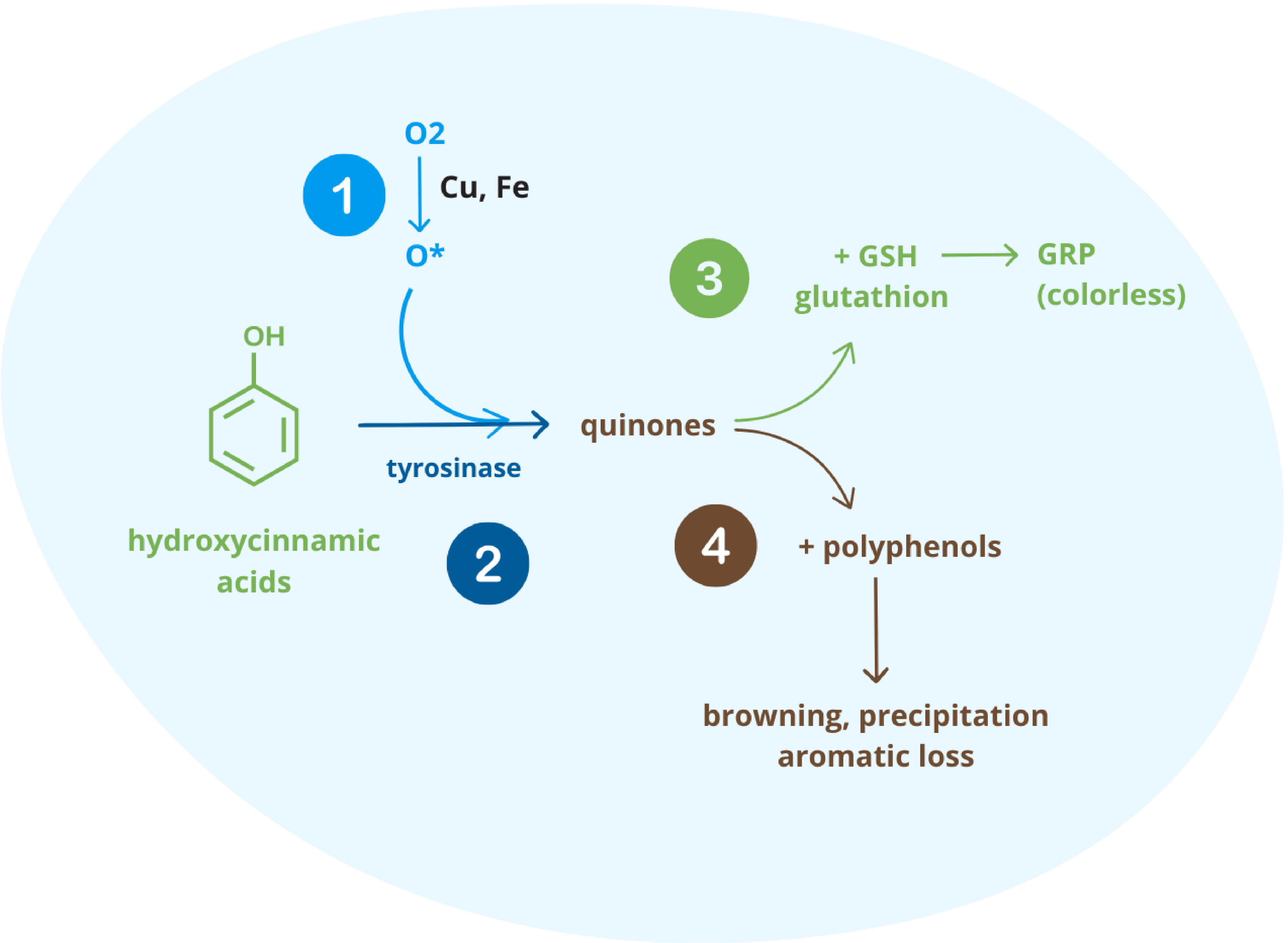


2. Levers for action to prevent must oxidation

The must oxidation mechanism

1. Dissolution of O_2 and conversion into molecular oxygen (catalysed by copper and iron)

2. Transformation of HA into quinones by tyrosinase enzymes, a highly reactive molecule

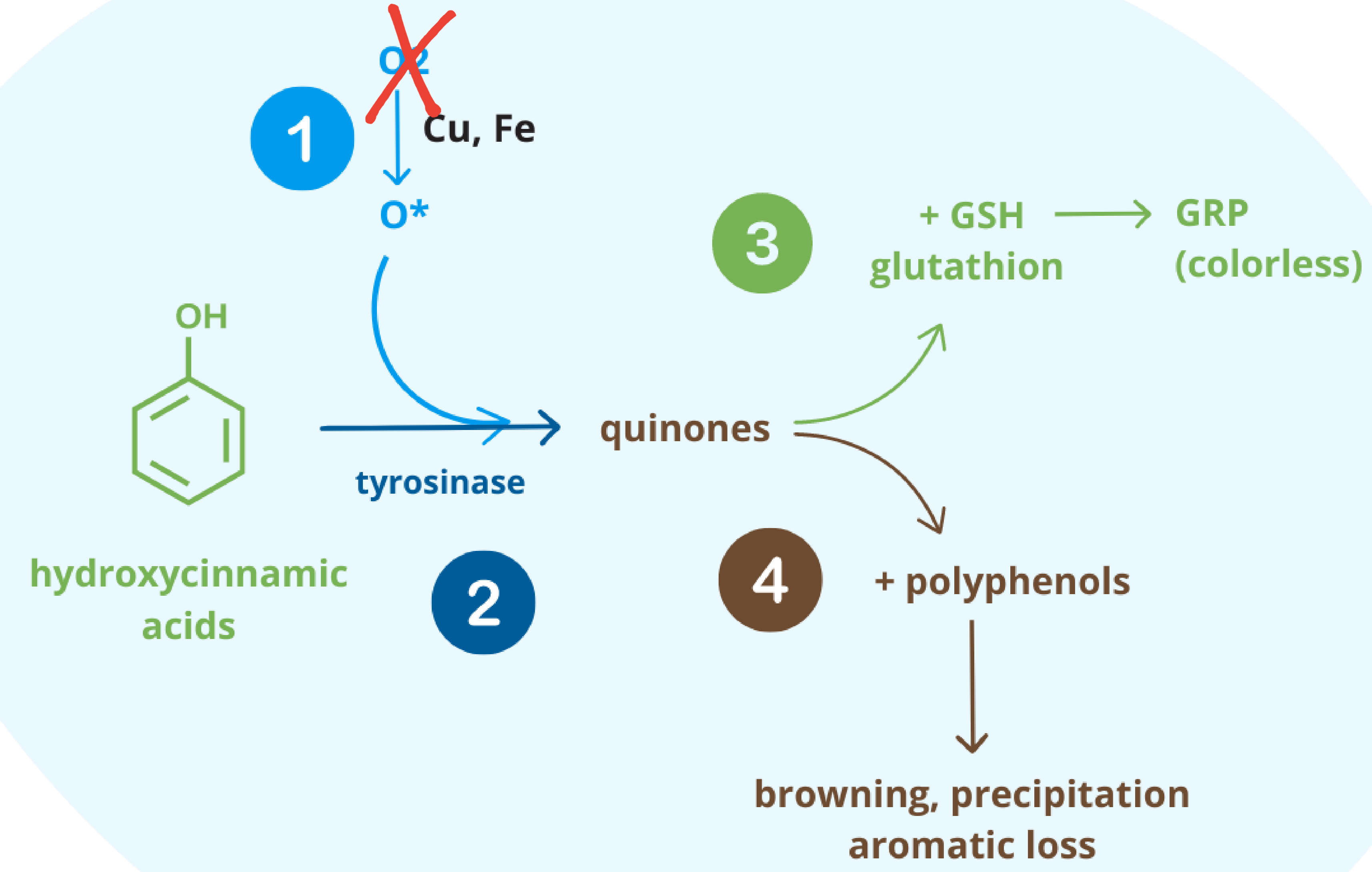


3. Consumption of quinones by glutathione to produce colourless GRP (Grape Reaction Product)

4. Glutathione depletion, oxidation of flavonoid polyphenols and aromas by quinones

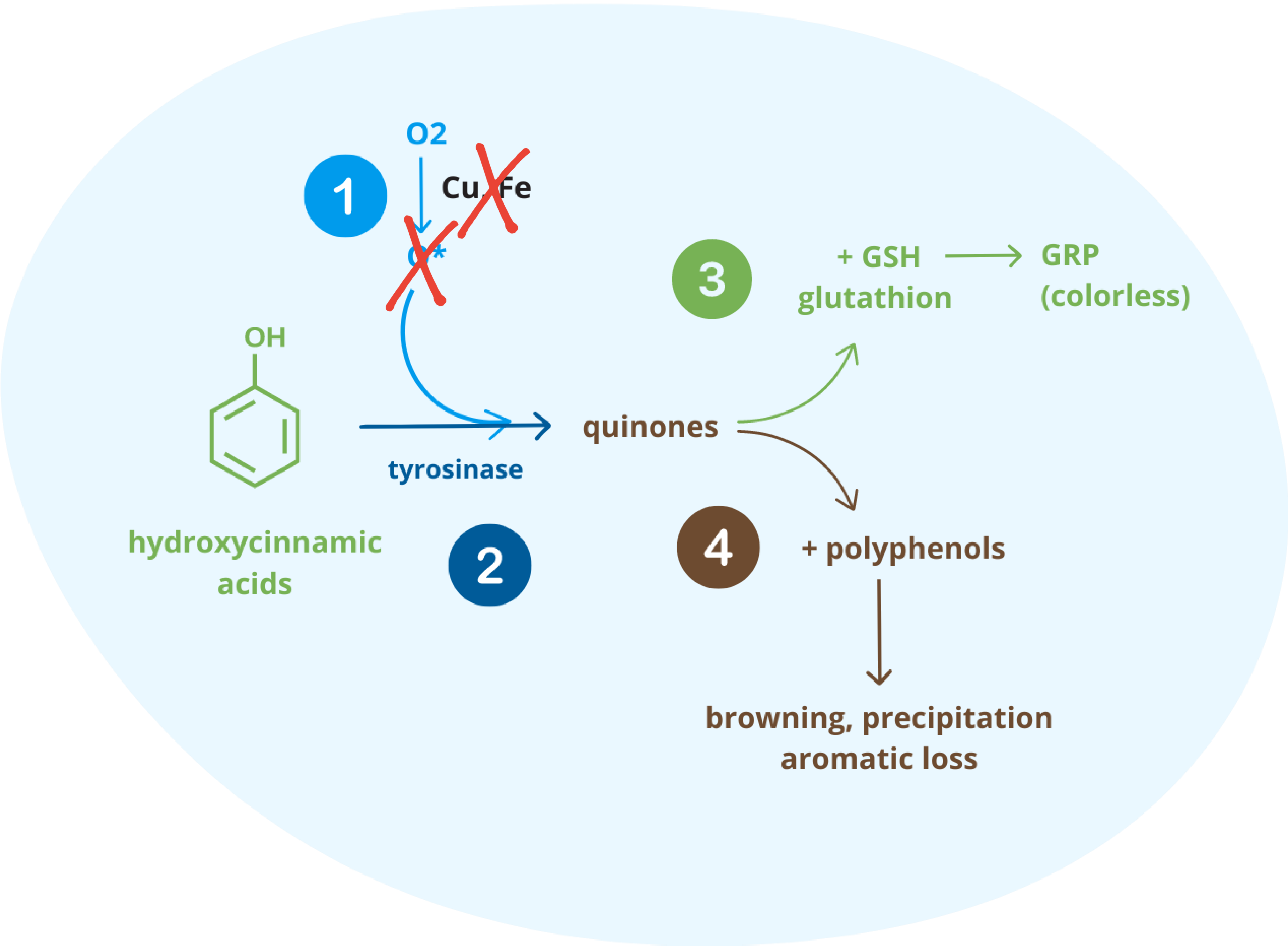
Strategies to prevent oxidation

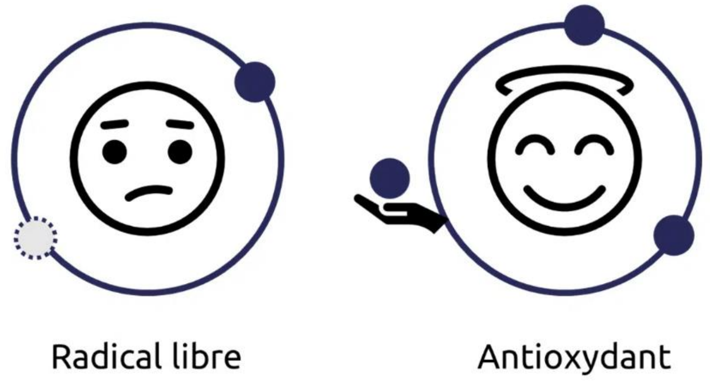
Lever 1 :
Prevent oxygen from dissolving in the must



Strategies to prevent oxidation

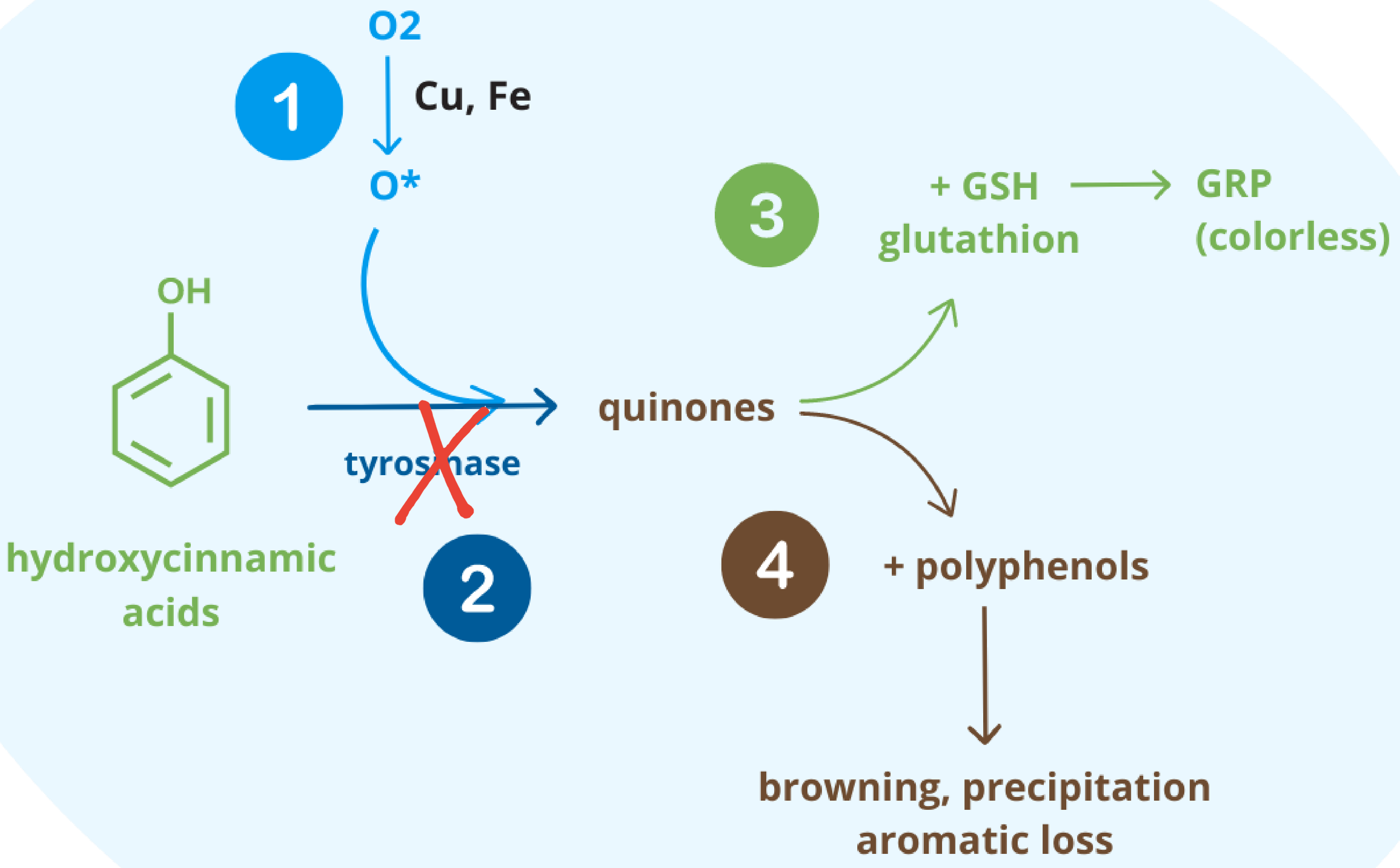
Lever 2 :
Neutralising free radicals





Strategies to prevent oxidation

Lever 3 :
Inhibiting oxidase enzymes



SO_2

< 12°C

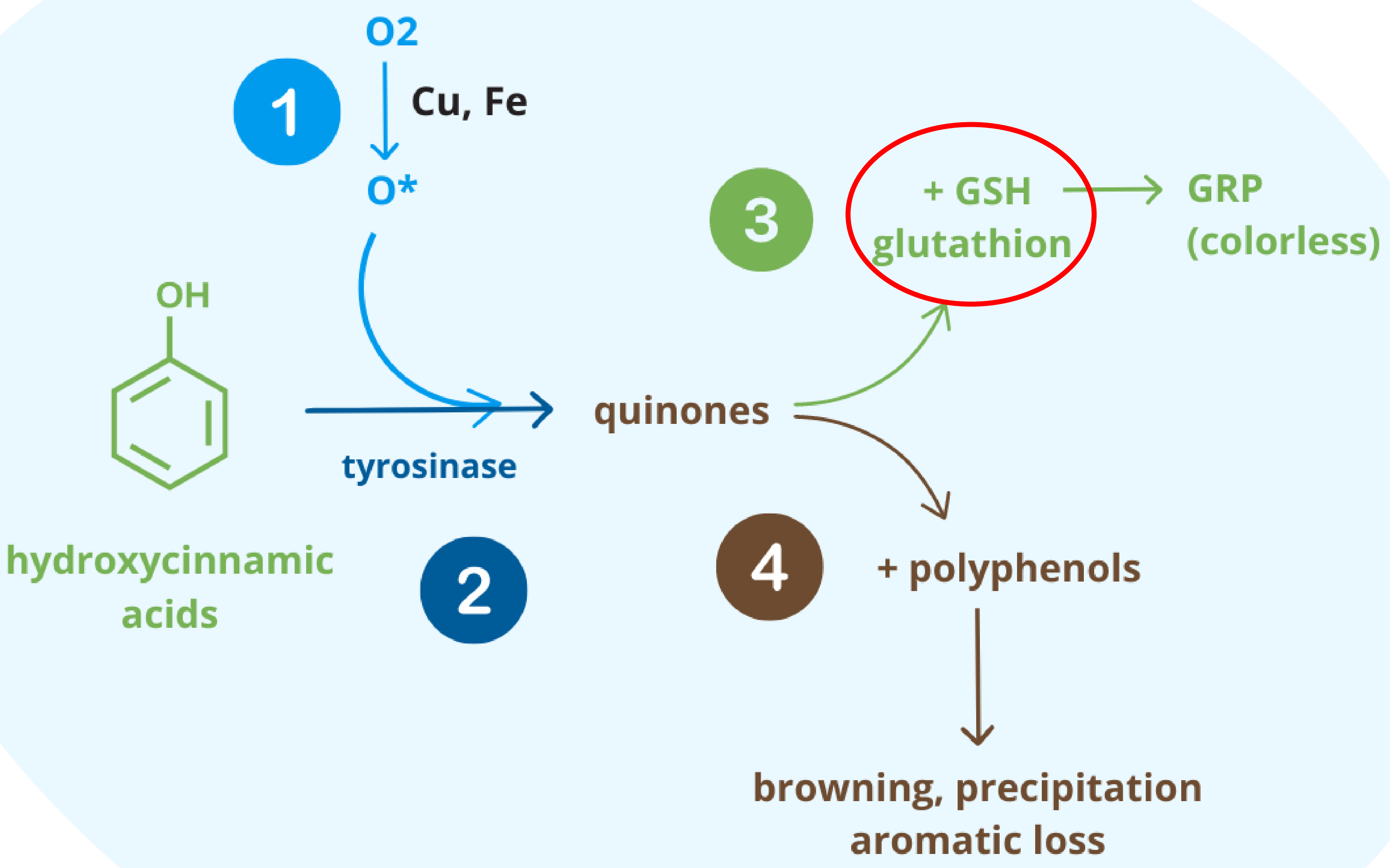
FULL PROTECT

ESSENTIAL ANTIOXIDANT

~~laccase~~

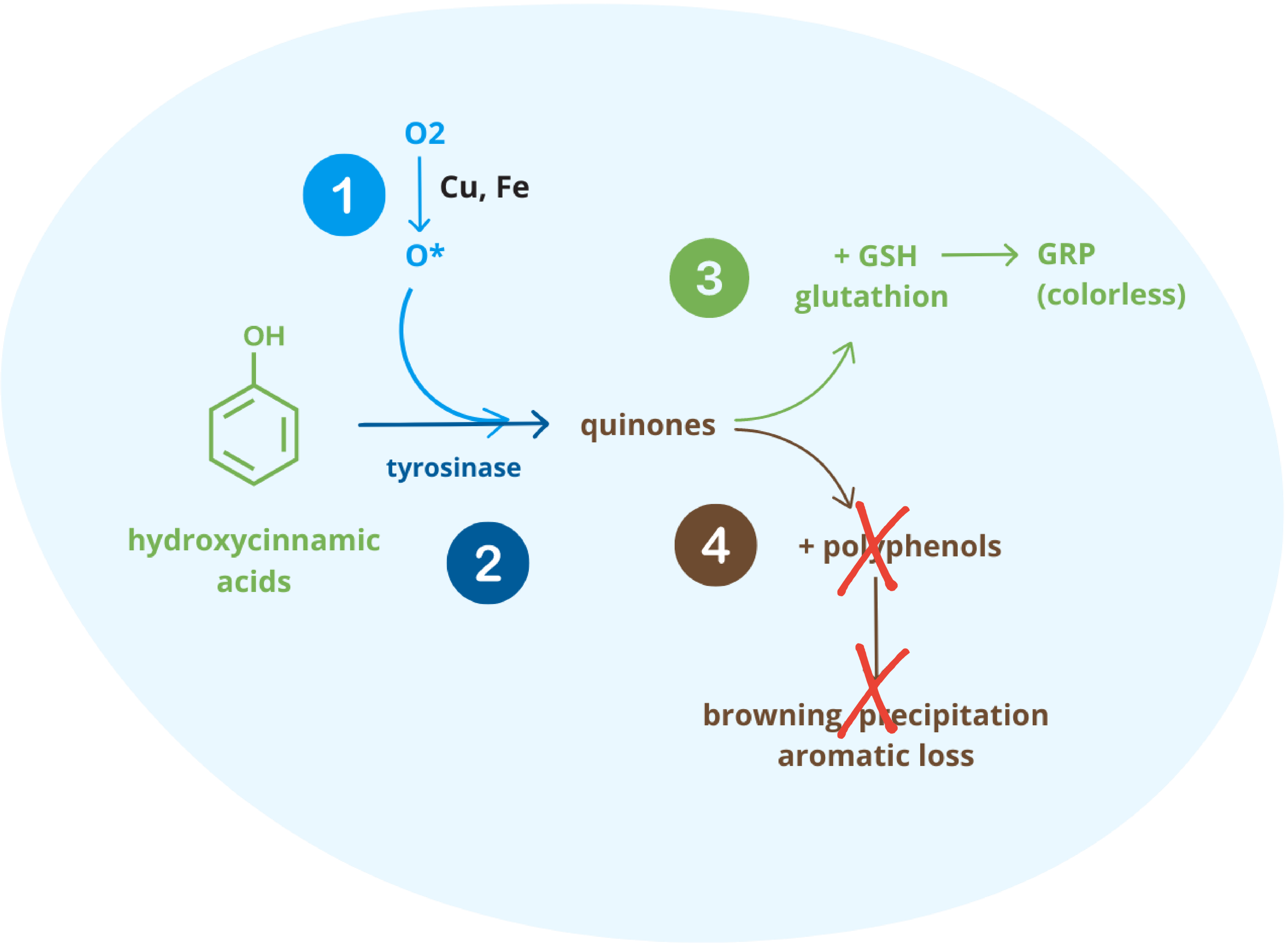
Strategies to prevent oxidation

Lever 4 :
Enriching the must with glutathione



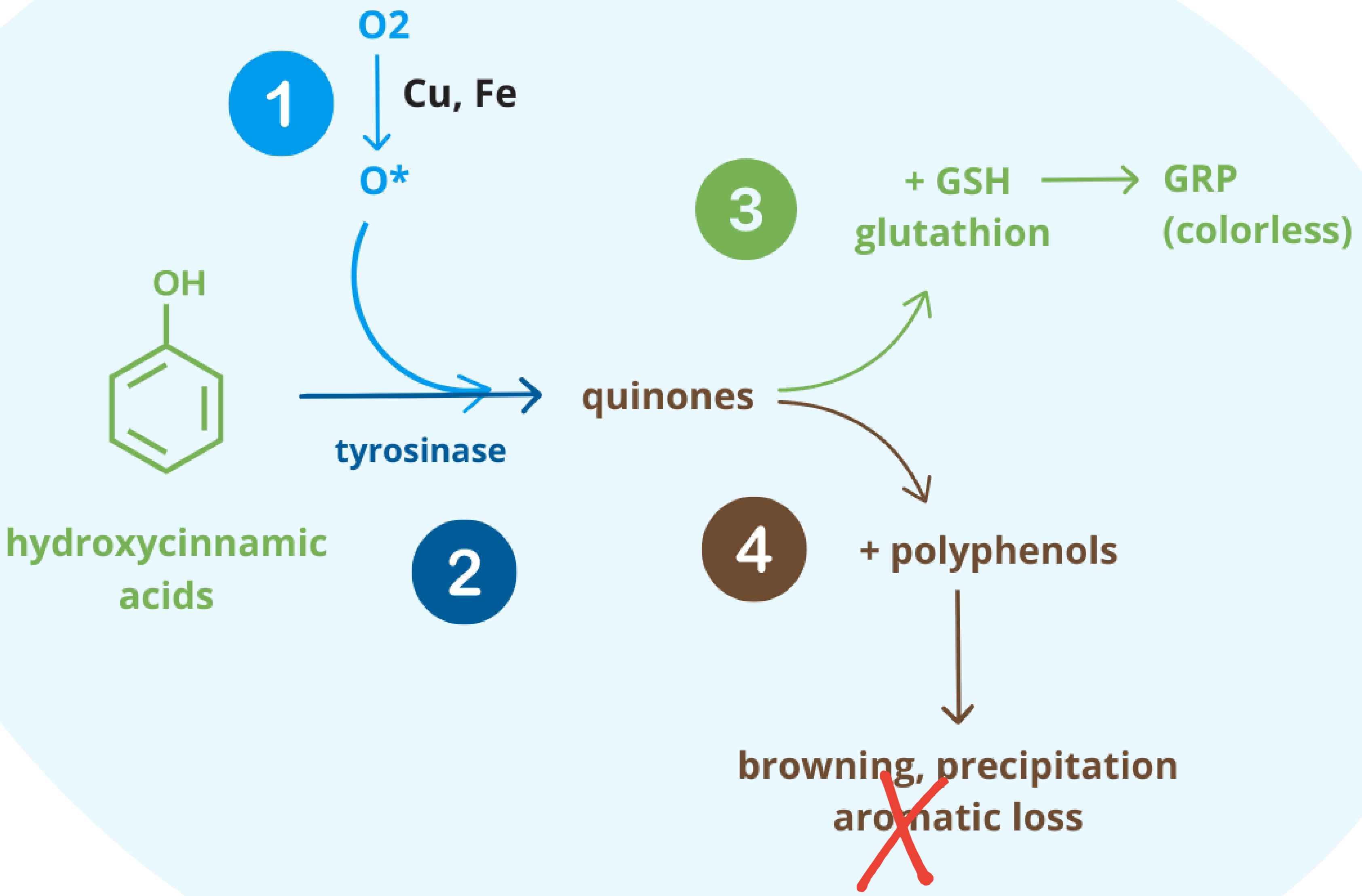
Glutarom Extra

Strategies to prevent oxidation



Lever 5 :
Eliminate oxidised and oxidisable polyphenols

Strategies to prevent oxidation



Lever 6 :
Stabilising aromas

Révélons votre différence

3. Combating oxidation in the cellar

Tools to combat oxidation

01 Oxygen

Preventive action against the dissolution of oxygen, and bioprotection with Calypso

02 Essential Antioxidant

Sacrificial tannin, neutralises free radicals, active against laccase

03 Full Protect

Tannins and inactivated yeast to combat oxidation and stabilise aromas

04 Glutarom Extra

Enriching the must with glutathione to protect aromas

05 Chitosan
Qi Fine / Qi Noox / Qi Up

Anti-free radical action
And elimination of oxidised and oxidisable polyphenols

06 Ultima Jump

Stabilisation of aromas before bottling

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01 | Preventing oxygen from dissolving in the must
Good preventive practices

Harvest

Limiting contact between the harvest and the air:

- Cover or closed tank
- Juice separation thanks to a double bottom

Avoid crushing and crushing the berries:

- Setting up the grape harvesting machine
- Limit the number and height of decanting operations

Act as **quickly as** possible:

- Transport time
- Early treatment of the harvest

Dry ice

Protecting the harvest as soon as it is picked and **lowering the temperature**

At the reception area, at the press, in the vat, ...

Pressing

Separate the first free run juice :

- Treat separately if oxidised, adapt doses

Use a **press inerted** with nitrogen

01 | Preventing oxygen from dissolving in the must
The use of dry ice

- **Forms of CO_2** to protect the harvest:
  - Dry snow (snow cannon)
  - Dry ice (compacted dry snow)

- **How much** dry ice **to use** :
  - Healthy grapes: 2kg for 10hL
  - On weathered grapes: 0.7kg for 1hL

    In practice, approximate doses




01 | Avoiding dissolved oxygen in the must
Measuring dissolved oxygen in the must

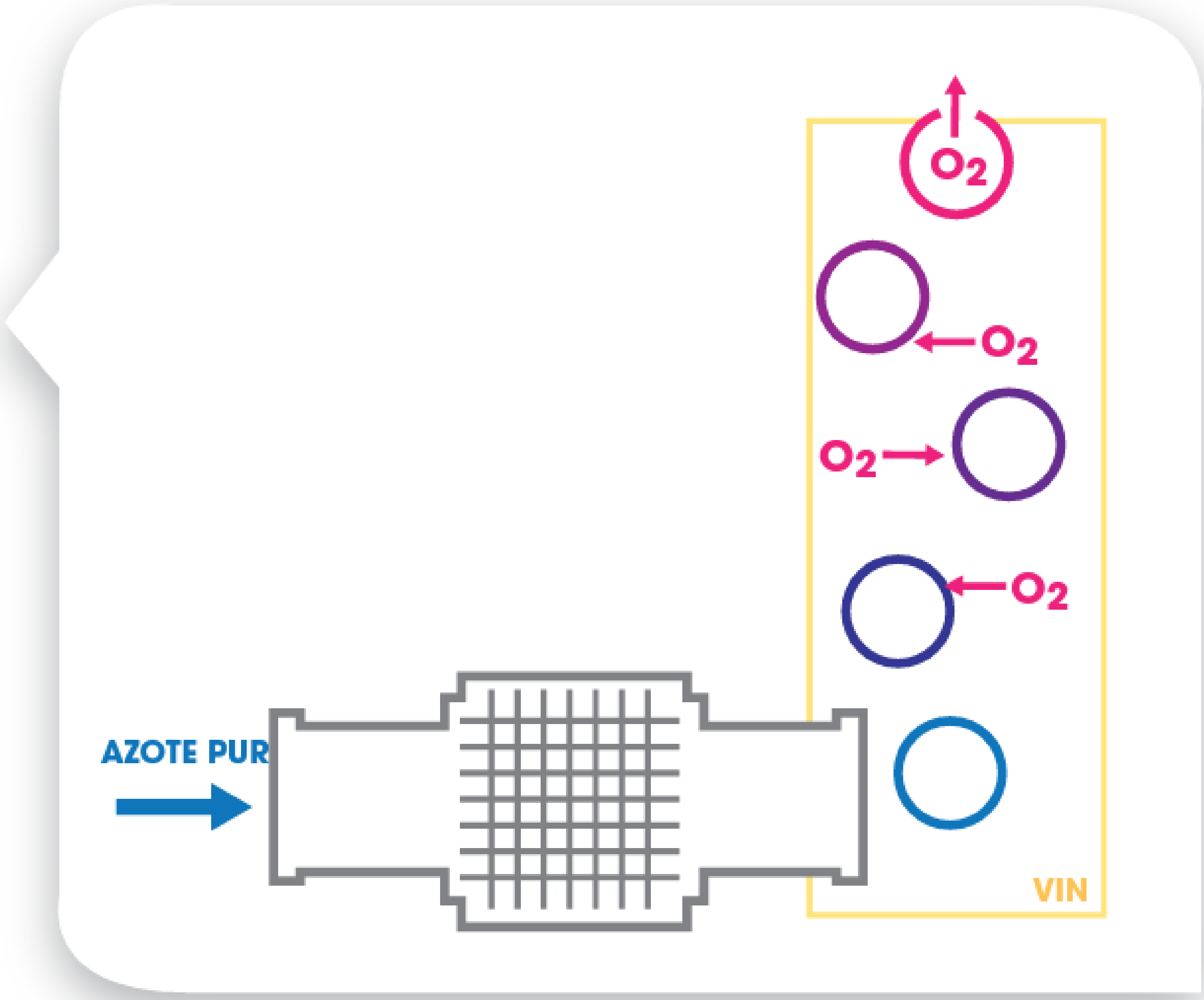
Measure to check that dissolved oxygen remains low:

- Measure in the must reception tank, after pressing
- Electrochemical measurements (or optical measurements)
- Values given in mg/L at 20°C
- Ideally, do not exceed 1 mg/L

01 | Avoid dissolving oxygen in the must
Deoxygenate with nitrogen if necessary

<u>Deoxygenate</u> if dissolved oxygen levels are too high.

- Injection of pure nitrogen bubbles into the juice, directly into the tank, via the bottom valve
- As they progress through the liquid, they become enriched with oxygen.
- Reduces O_2 content to less than 1 mg/L



01 | Preventing oxygen from dissolving in the must
Deoxygenation equipment

01 | Avoiding the dissolution of oxygen in the must
Bioprotection

- Non-fermentative bioprotection yeast
- Naturally consumes dissolved oxygen
- Reveals and protects aromas

Tools to combat oxidation

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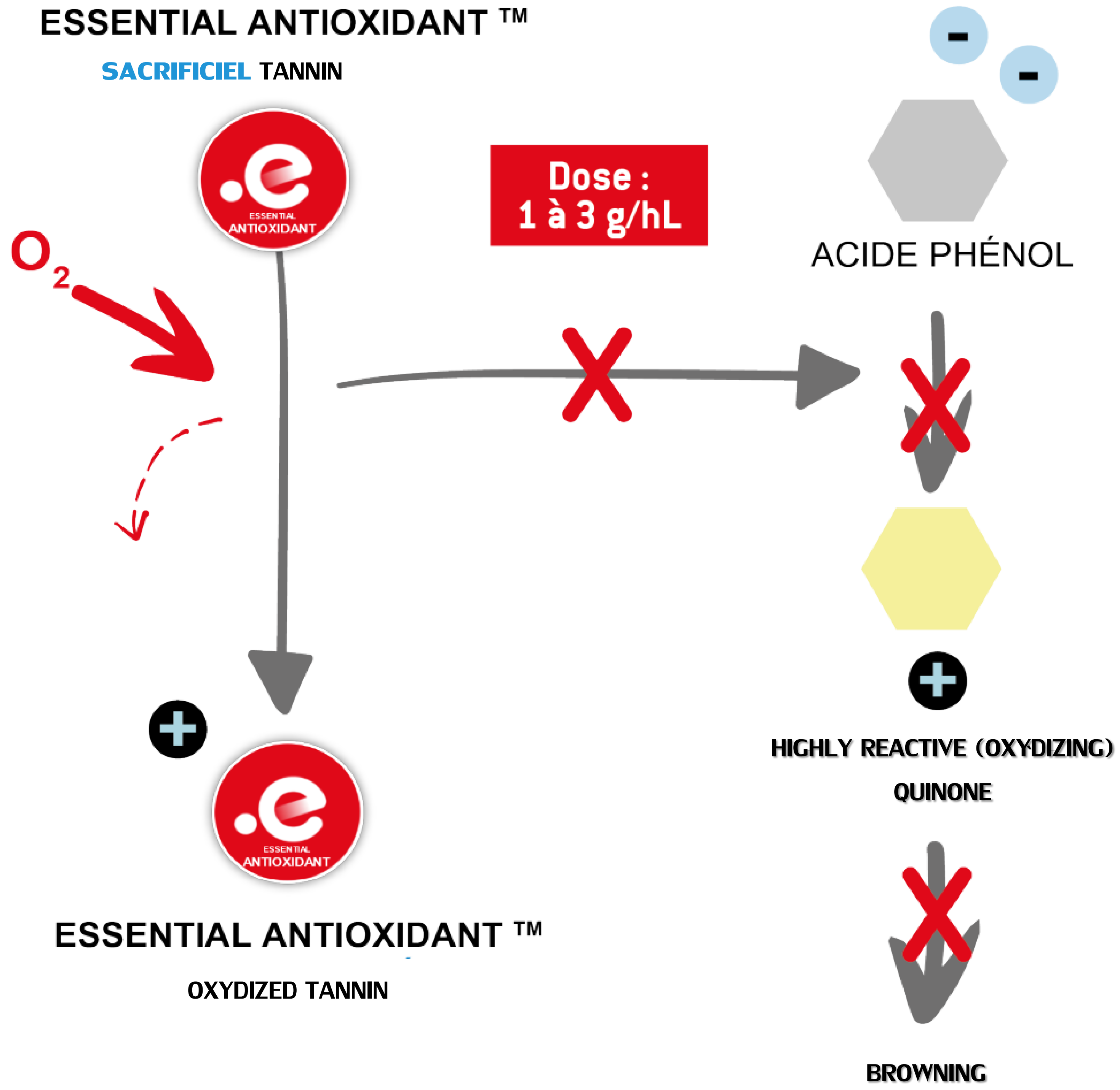
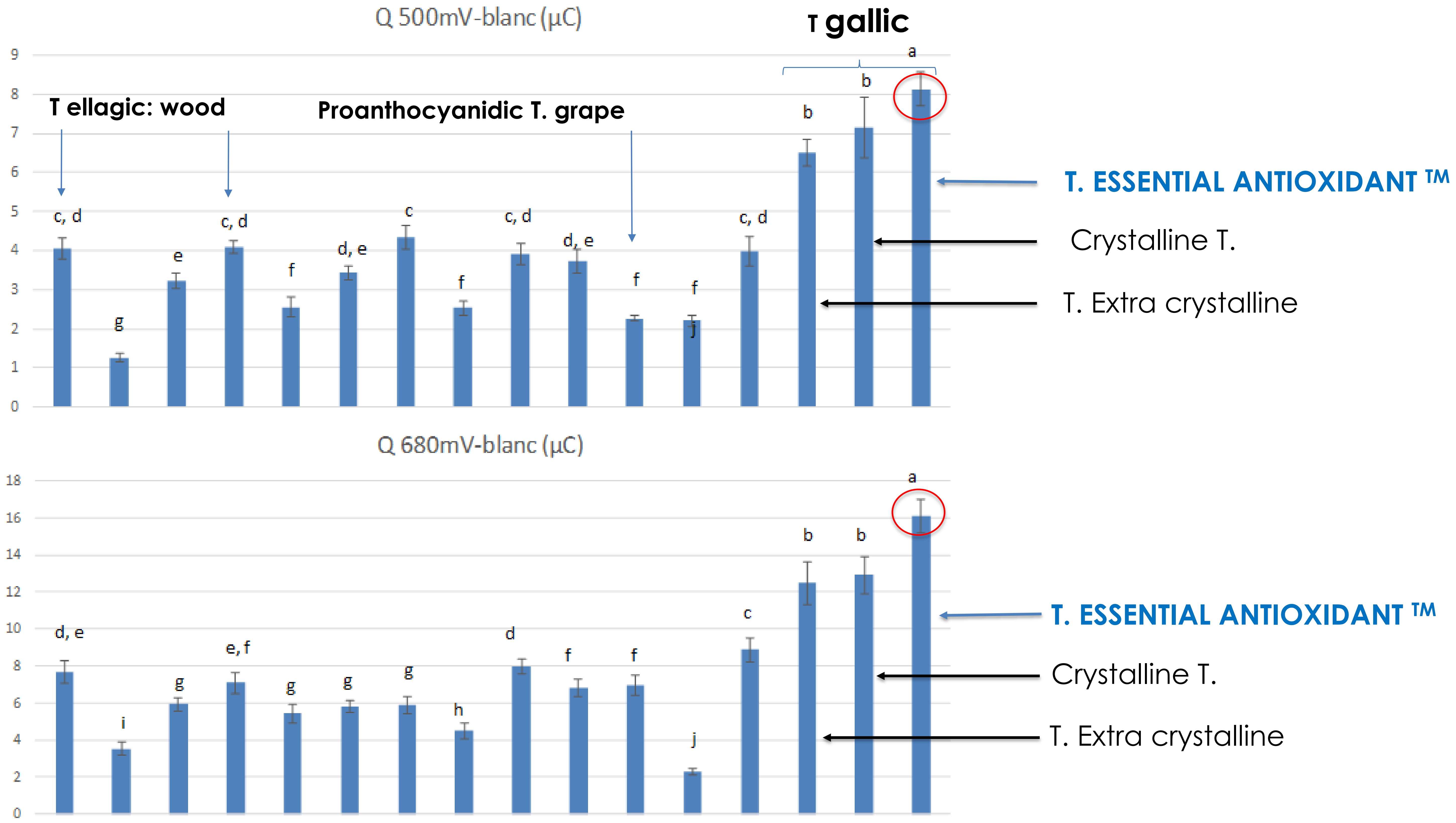
Stabilisation of aromas before bottling

02 | ESSENTIAL ANTIOXIDANT



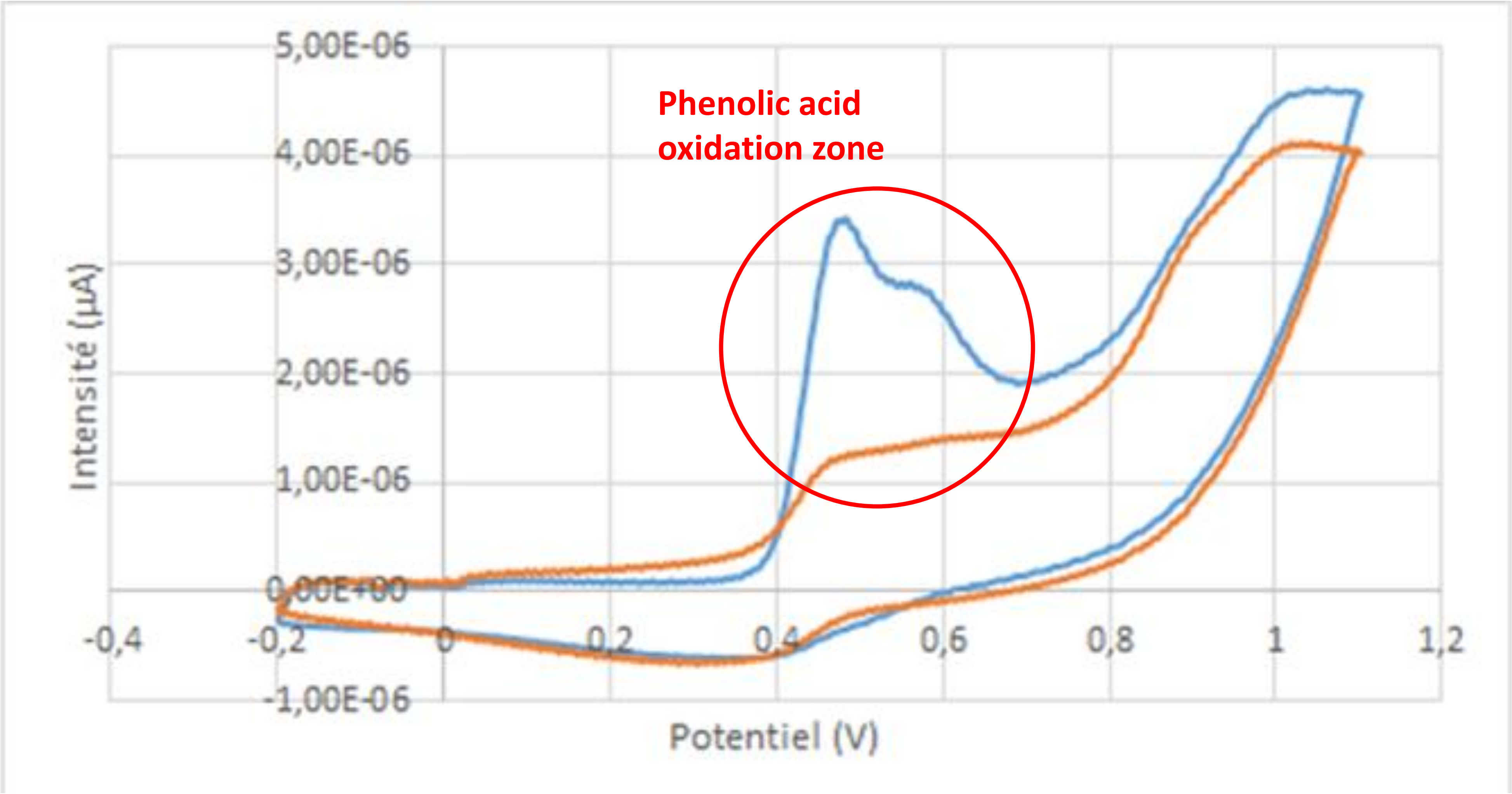
Strong antioxidant (sacrificial tannin) and **anti-laccase** properties

02 | ESSENTIAL ANTIOXIDANT
The antioxidant capacity of tannins



02 | ESSENTIAL ANTIOXIDANT
Additional characterisation of antioxidant action

Determination of total antioxidant activity



TEMOIN SAUVIGNON BLANC

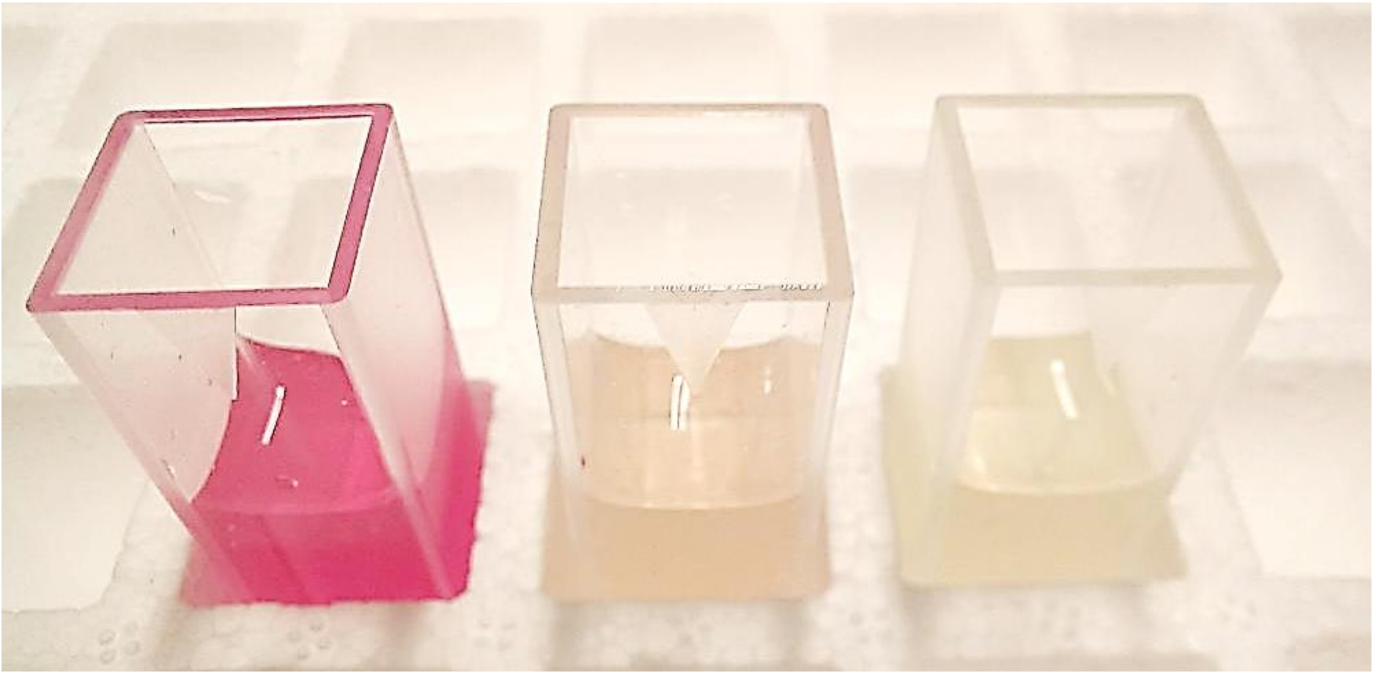
5 g/hl ESSENTIAL ANTIOXIDANT

02 | ESSENTIAL ANTIOXIDANT
Characterisation of anti-laccase action

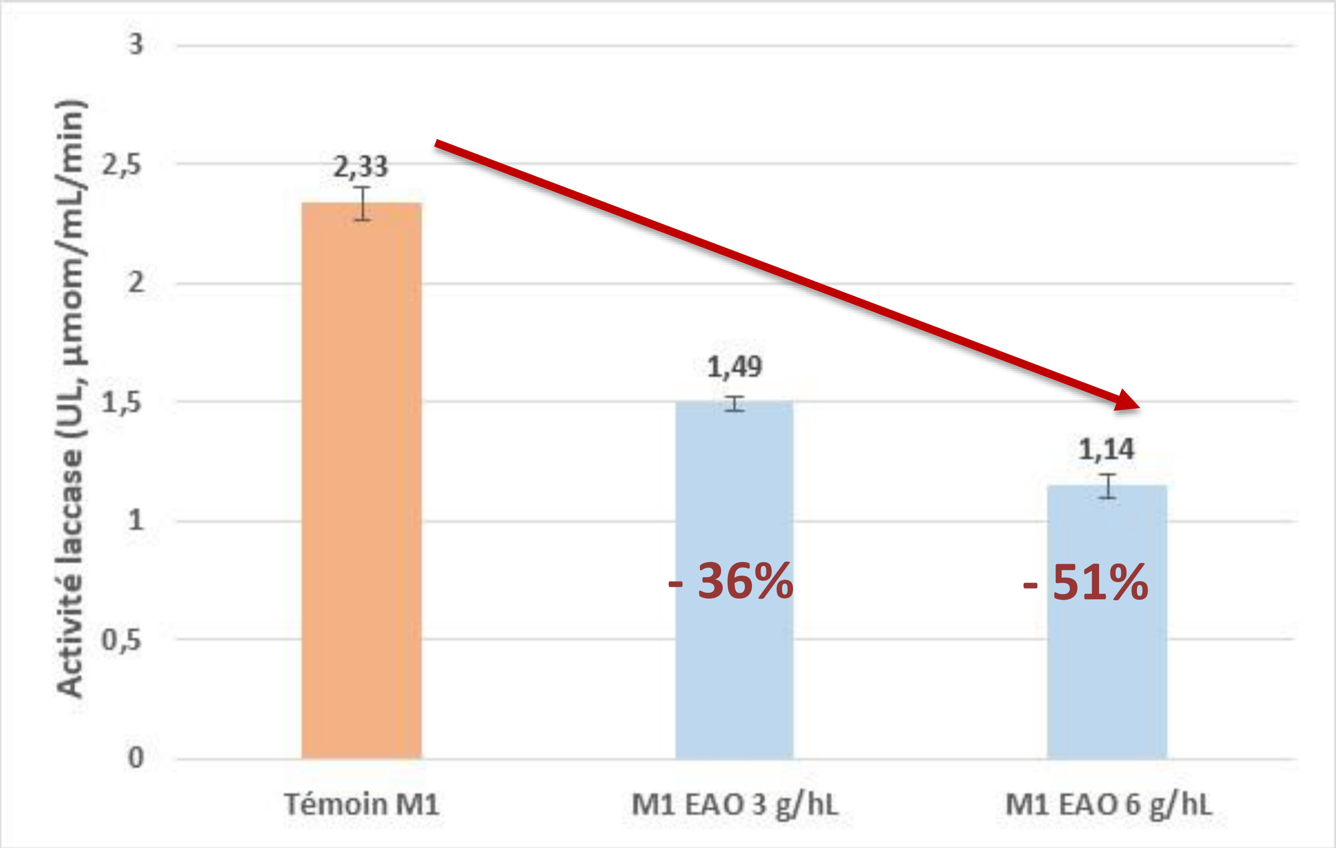
Determination of laccase activity using a laccase test :

Laccase activity = (46.15 × ΔA)/min (UL, µmol/mL/min)

Grassin and Dubourdieu, 1986, and Vigault et al., 2019



Control | + EAO 3 g/hL | + EAO 6 g/hL



Pinot Meunier trial, 25 to 30% botrytised grapes, gluconic acid = 0.31 g/L

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Stabilisation of aromas before bottling

03 | FULL PROTECT
A gallic tannin, blended with inactivated yeast

- Overall reduction in oxygen sensitivity

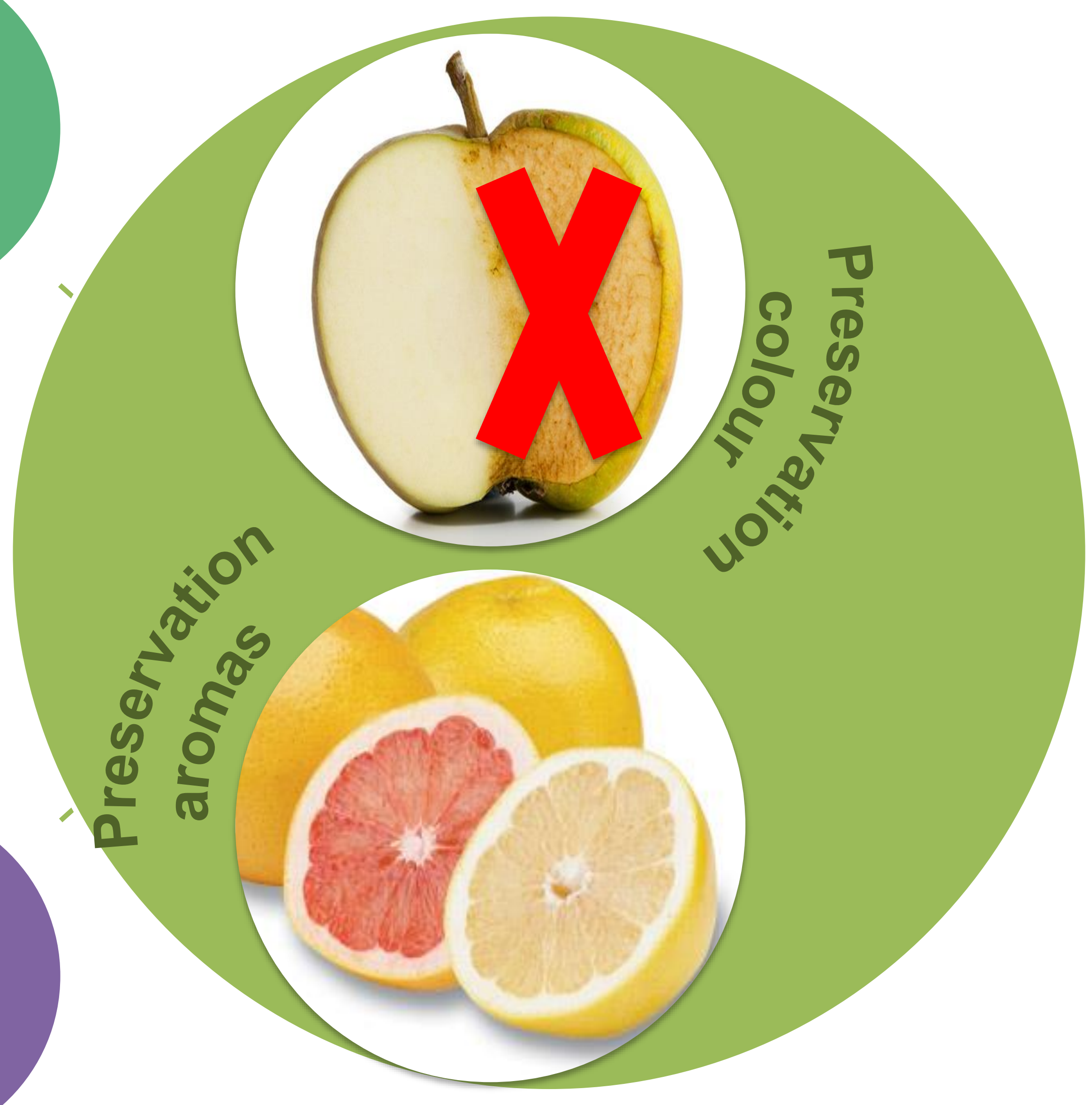
Overall **reductive power of** yeast

- **<u>Release of mannoproteins</u>** and stabilisation of aromas just after their formation

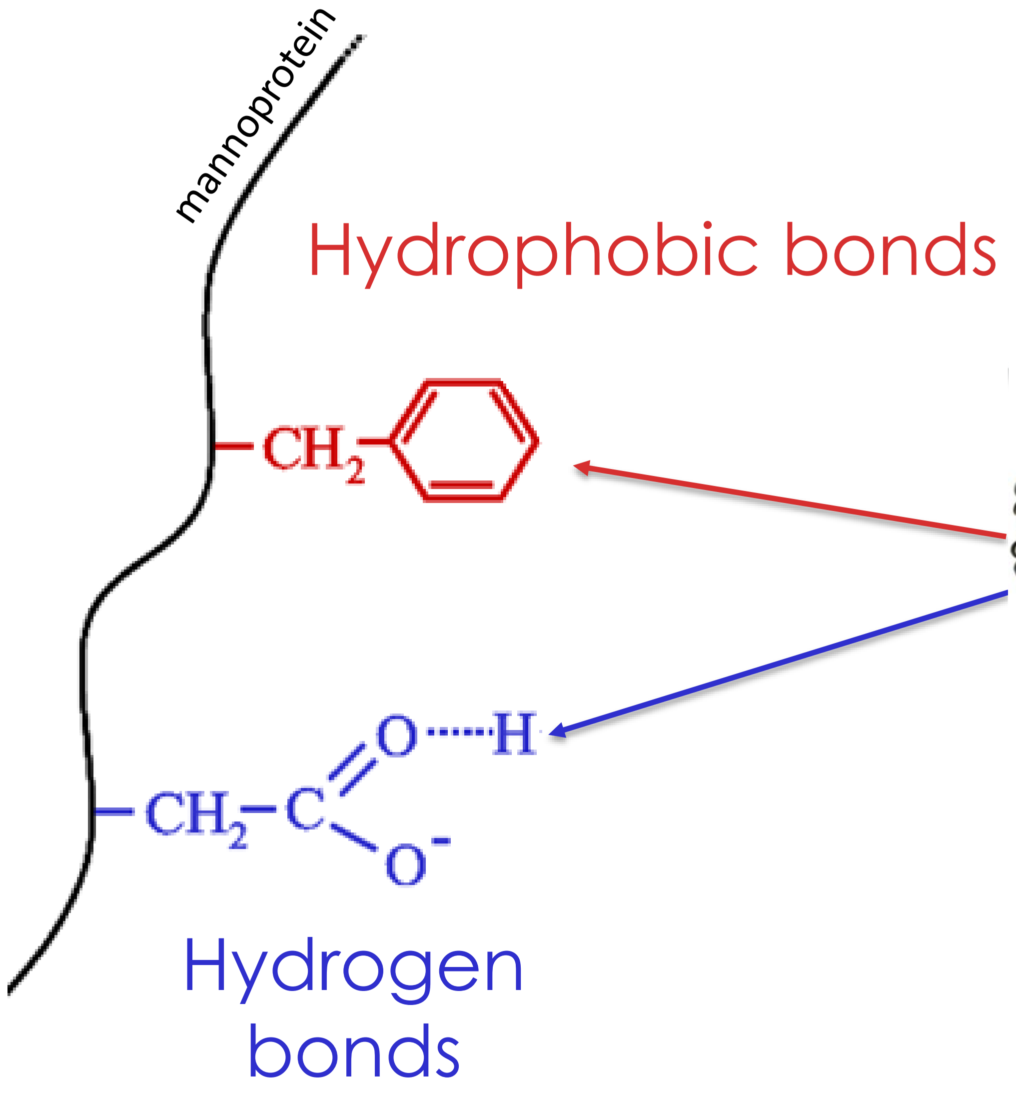
Release of **soluble macro-molecules**

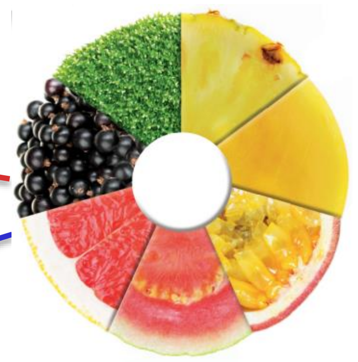
- Protection against oxidation of the phenolic acids in grapes

Synergy with a specific **tannin**



03 | FULL PROTECT
The benefits of mannoproteins for flavour stabilisation

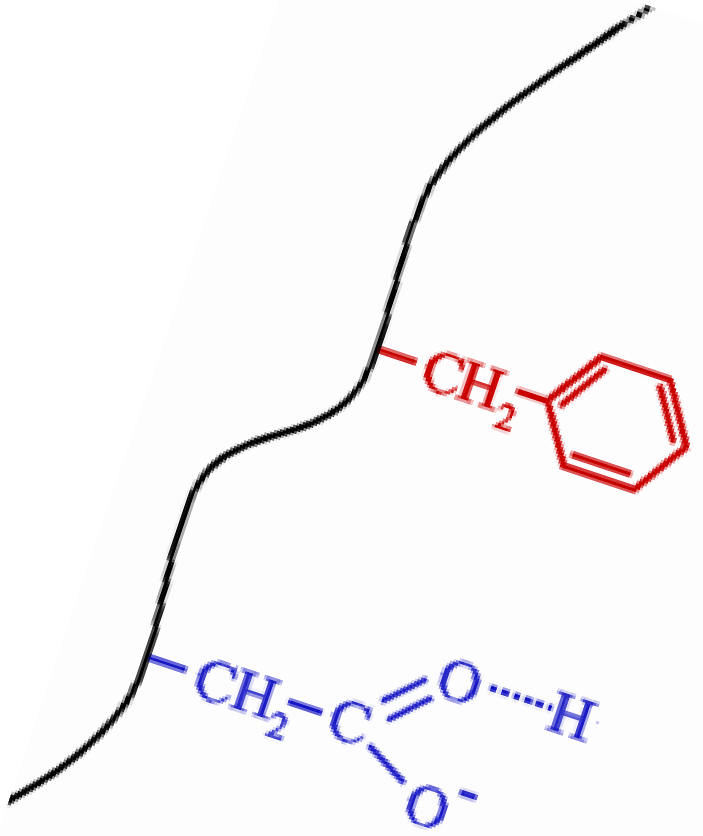




Flavours

Less loss through volatilisation
Less vulnerability to oxygen

03 | FULL PROTECT
When and in what form should mannoproteins be added?



Pre-fermentation phase

Inactivated yeast rich in mannoproteins

Before bottling

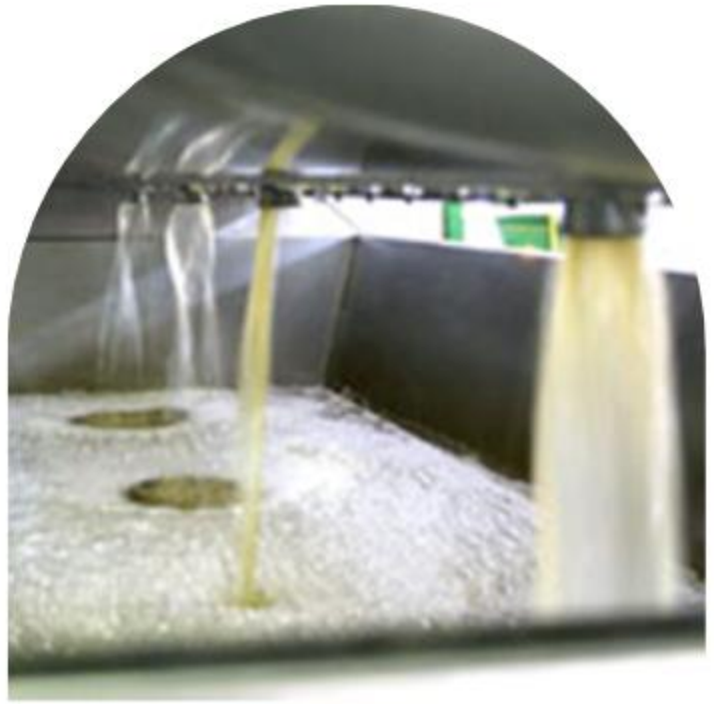
Formulation of yeast mannoproteins

03 | FULL PROTECT
How do I use it?

Dose d'emploi indicative :
30 g/hL

All at once or in fractions:

FULL PROTECT



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06 **Ultima Jump**

Stabilisation of aromas before bottling

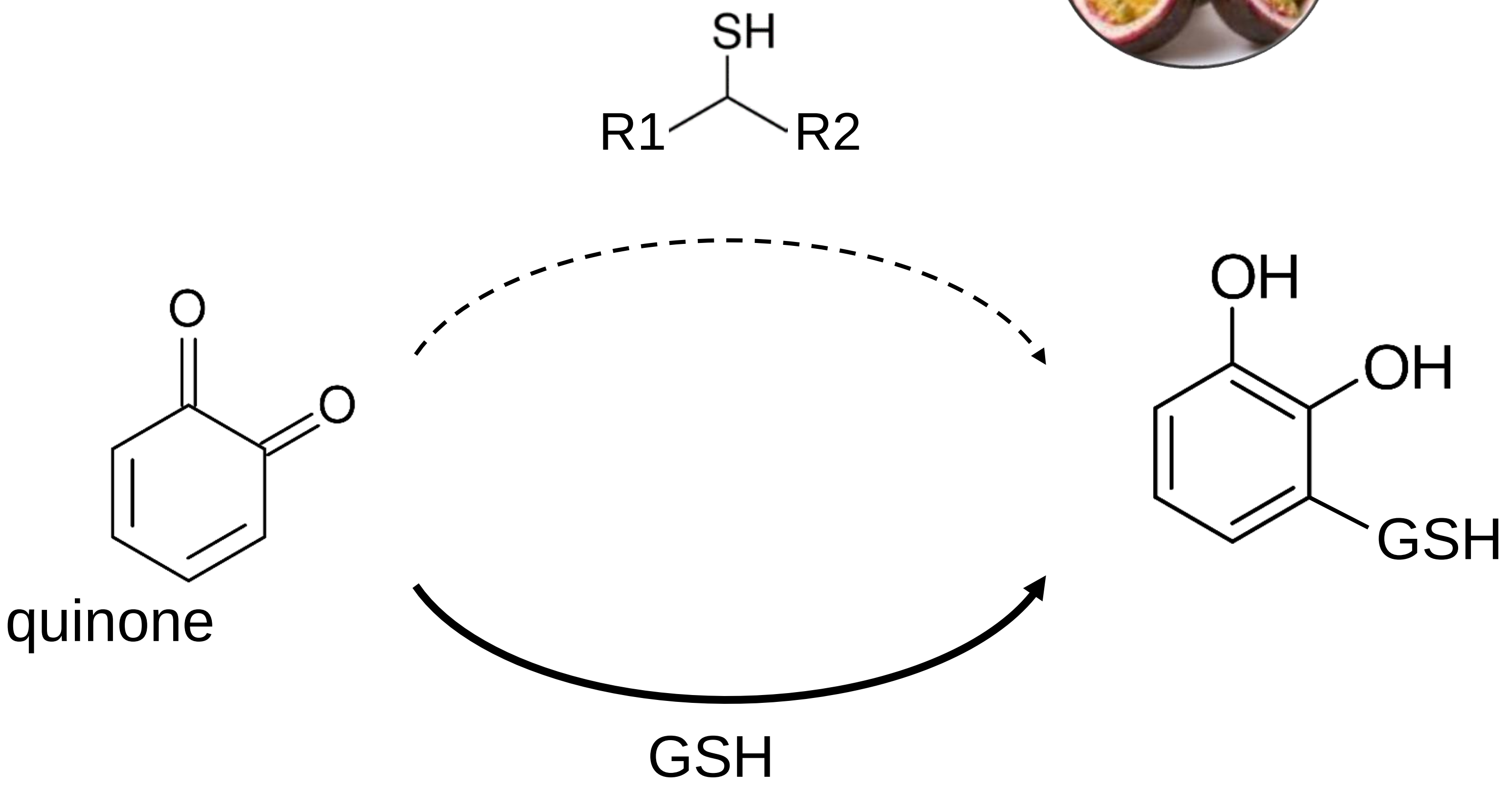
04 | GLUTAROM EXTRA
Protecting aromas as soon as they are formed

GLUTAROM EXTRA

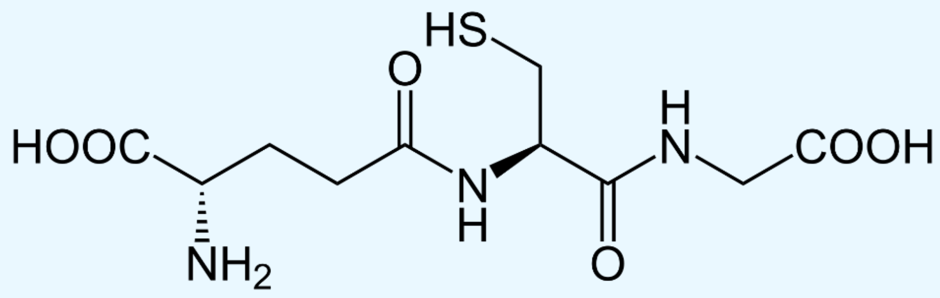
- Inactivated yeast
- High glutathione concentration
- Addition to must, or at the start of AF
- Long-lasting protection

04 | GLUTAROM EXTRA
Protect flavours by combating quinones

Fruity varietal thiols
nucleophiles



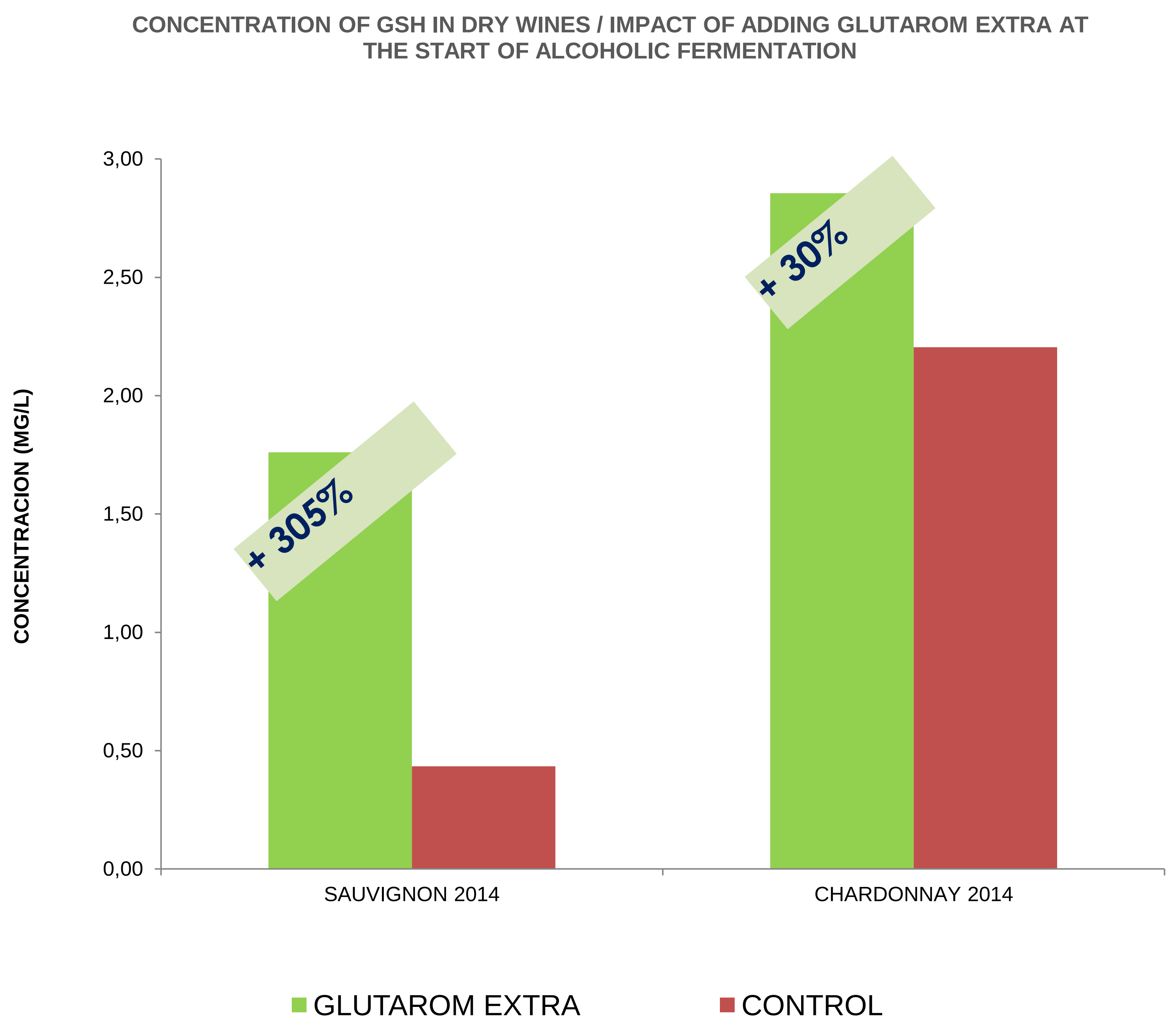
Nucleophilic
competition with
glutathione



GSH

The addition of inactivated yeast at the <u>start of </u>alcoholic <u>fermentation</u> produces the highest concentrations of GSH in finished wines.

04 | GLUTAROM EXTRA
Long-lasting protection



Tools to combat oxidation

01 **Oxygen**
Preventive action against the dissolution of oxygen, and bioprotection with Calypso

02 **Essential Antioxidant**
Sacrificial tannin, neutralises free radicals, active against laccase

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Tannins and inactivated yeast to combat oxidation and stabilise aromas

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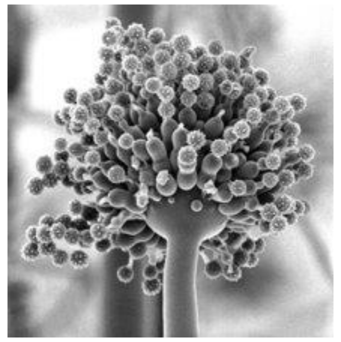
Enrichment of the must with glutathione, to protect aromas

05 **Chitosan**
Qi Fine / Qi Noox / Qi Up
Anti-free radical action
And elimination of oxidised and oxidisable polyphenols

06 **Ultima Jump**
Stabilisation of aromas before bottling

05 | Oenological chitosan
Fungal origin

Fungal origin chitosan with high charge density



➔ 2 specific types of chitosan which differ in their chemical configuration, degree of acetylation and molecular weight

Anti-microbiological chitosan

Biological action

Dismemberment of the cellular membrane (*Brettanomyces*, lactic and acetic acid bacteria)

Physical action

Clarifying:
High capacity and speed of floculation and sedimentation

Fining chitosan

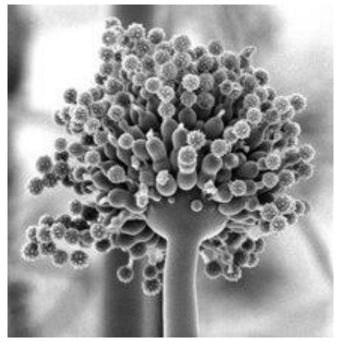
Chemical action

Anti-oxidant :

- Interactions with polyphenols
- Metal trapping (Cu, Fer)
- Anti-radical properties

05 | Oenological chitosan

Fungal origin chitosan with high charge density



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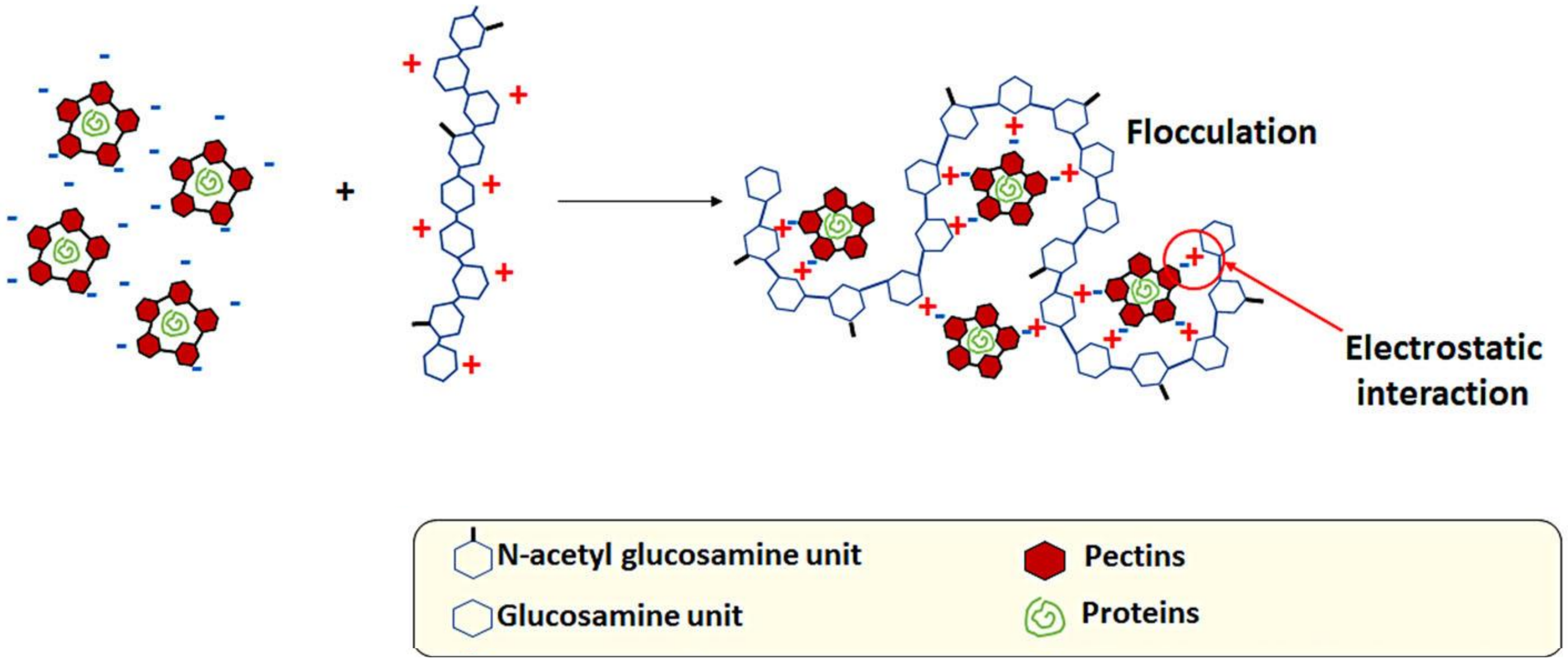
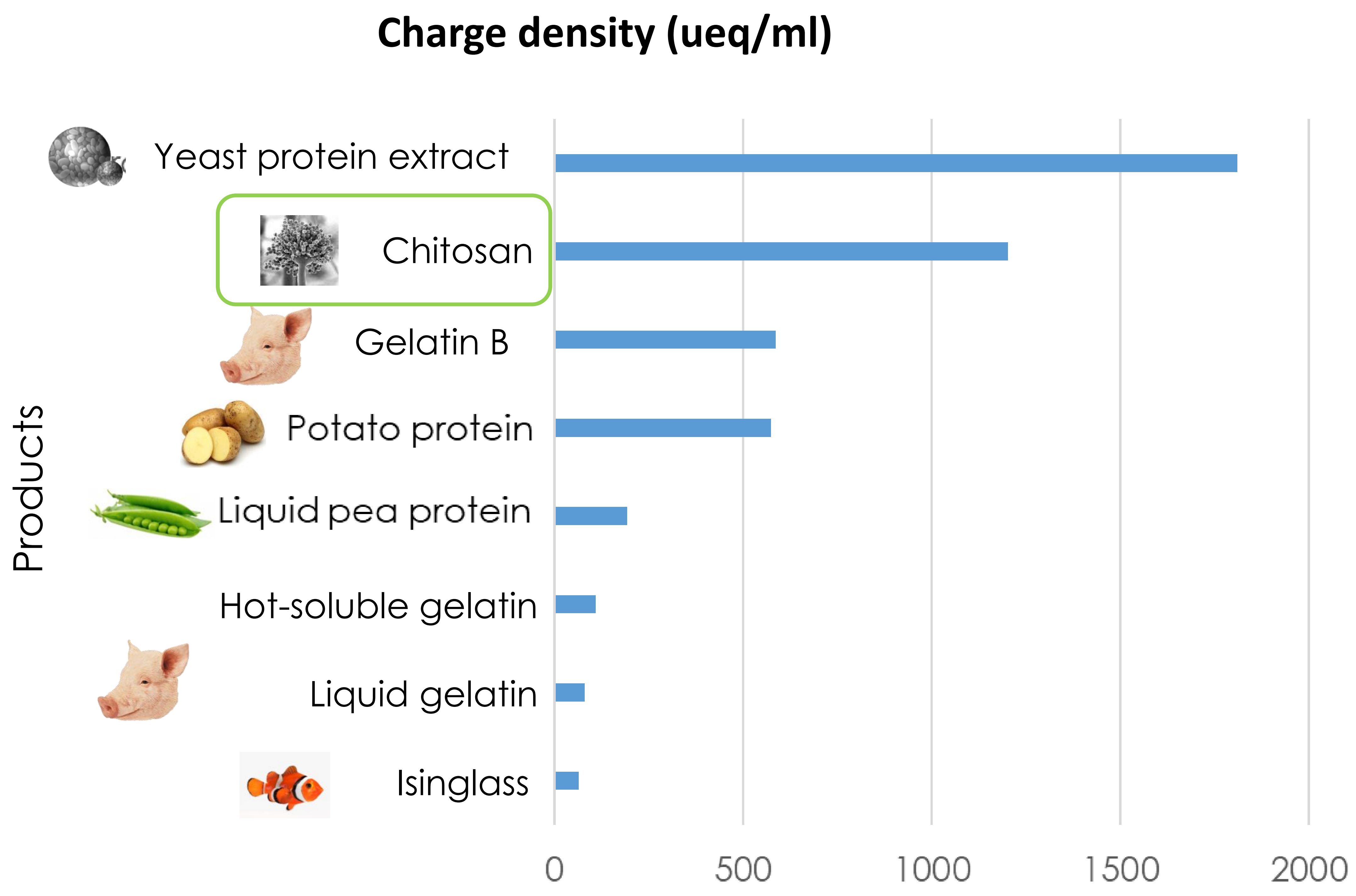
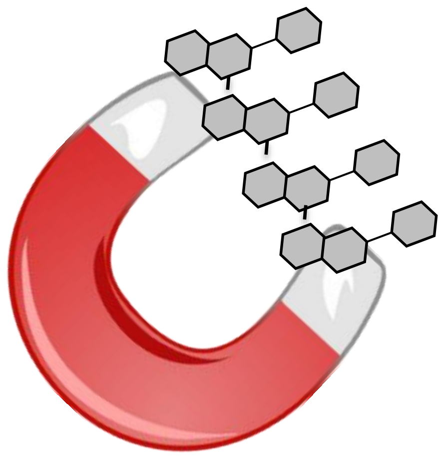
05 | Oenological chitosan
Physical action



According to Castro & al, C.R.F.S.N (2021)

05 | Oenological chitosan
Charge densities of different fining agent





05 | Oenological chitosan

Fungal origin chitosan with high charge density

➔ 2 specific types of chitosan which differ in their chemical configuration, degree of acetylation and molecular weight

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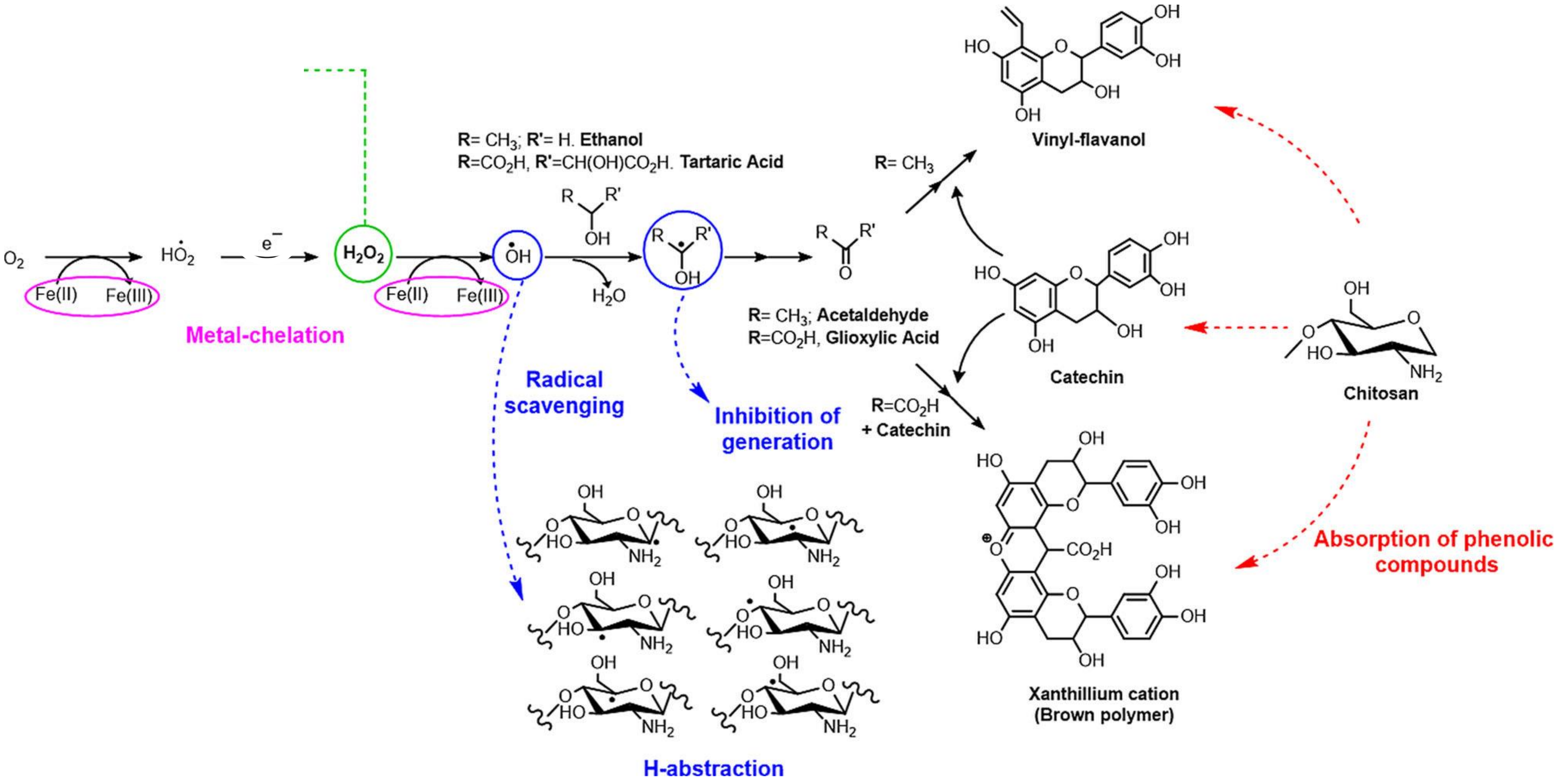
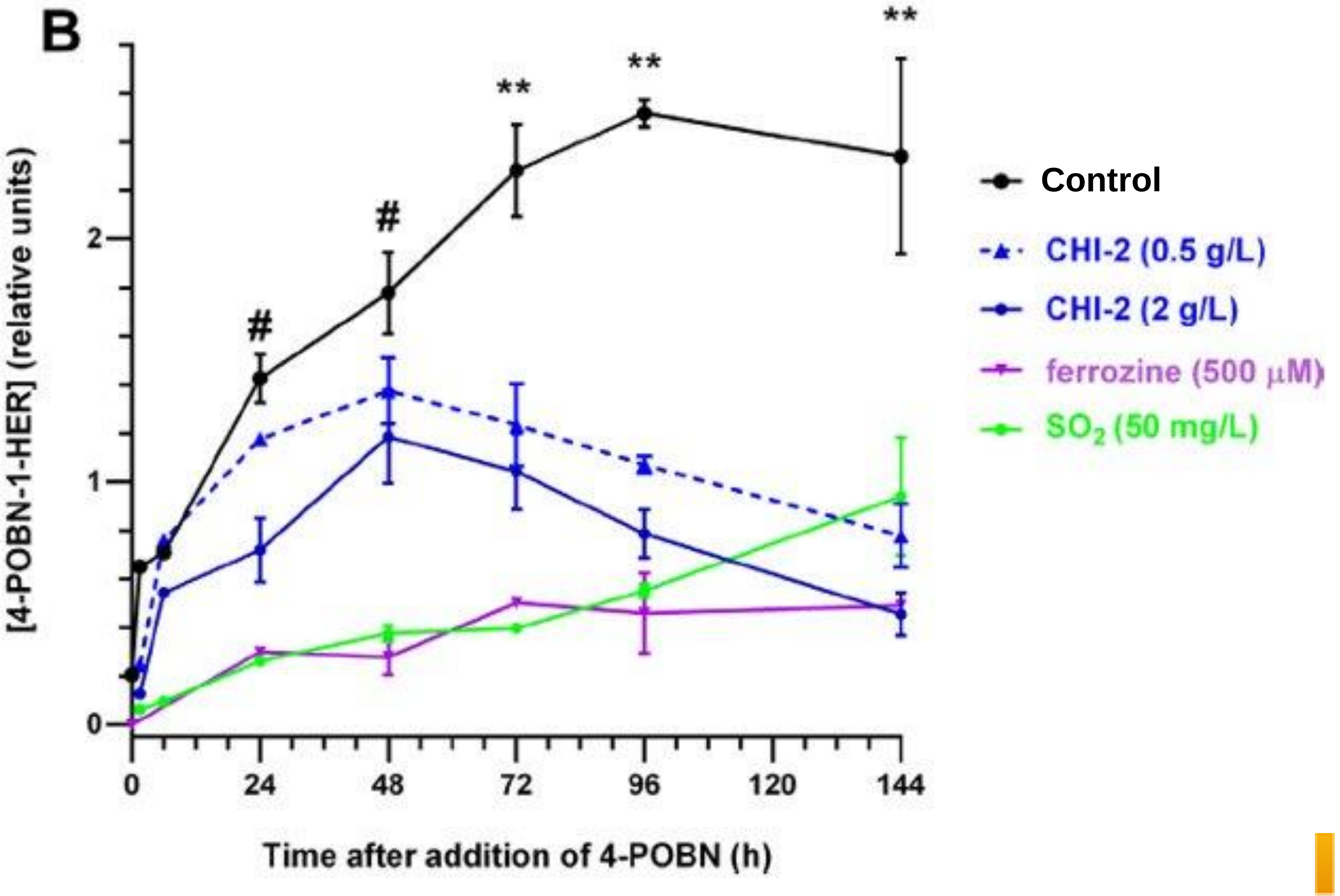
05 | Oenological chitosan
Chemical action



05 | Oenological chitosan
Why use chitosan to combat free radicals?

EPR (electron paramagnetic resonance) :
Monitoring the presence of free radicals



Chardonnay (Basic wine 2016 - SO_2 < 5mg/L)

- SO_2: initially active, then consumed and much less active
- Chitosan: **lasting effectiveness over time**

Institut Chimie Radicalaire

Chitosan as an antioxidant alternative to sulphites in oenology: EPR investigation of inhibitory mechanisms

Antonio Castro Marín[a,b], Marcel Culcasi[a,*], Mathieu Cassien[a], Pierre Stocker[a], Sophie Thétiot-Laurent[a], Bertrand Robillard[c], Fabio Chinnici[b], Sylvia Pietri[a]

[a] Aix Marseille Univ, CNRS, ICR, Marseille, France
[b] Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy
[c] Institut Oenologique de Champagne, Epernay, France

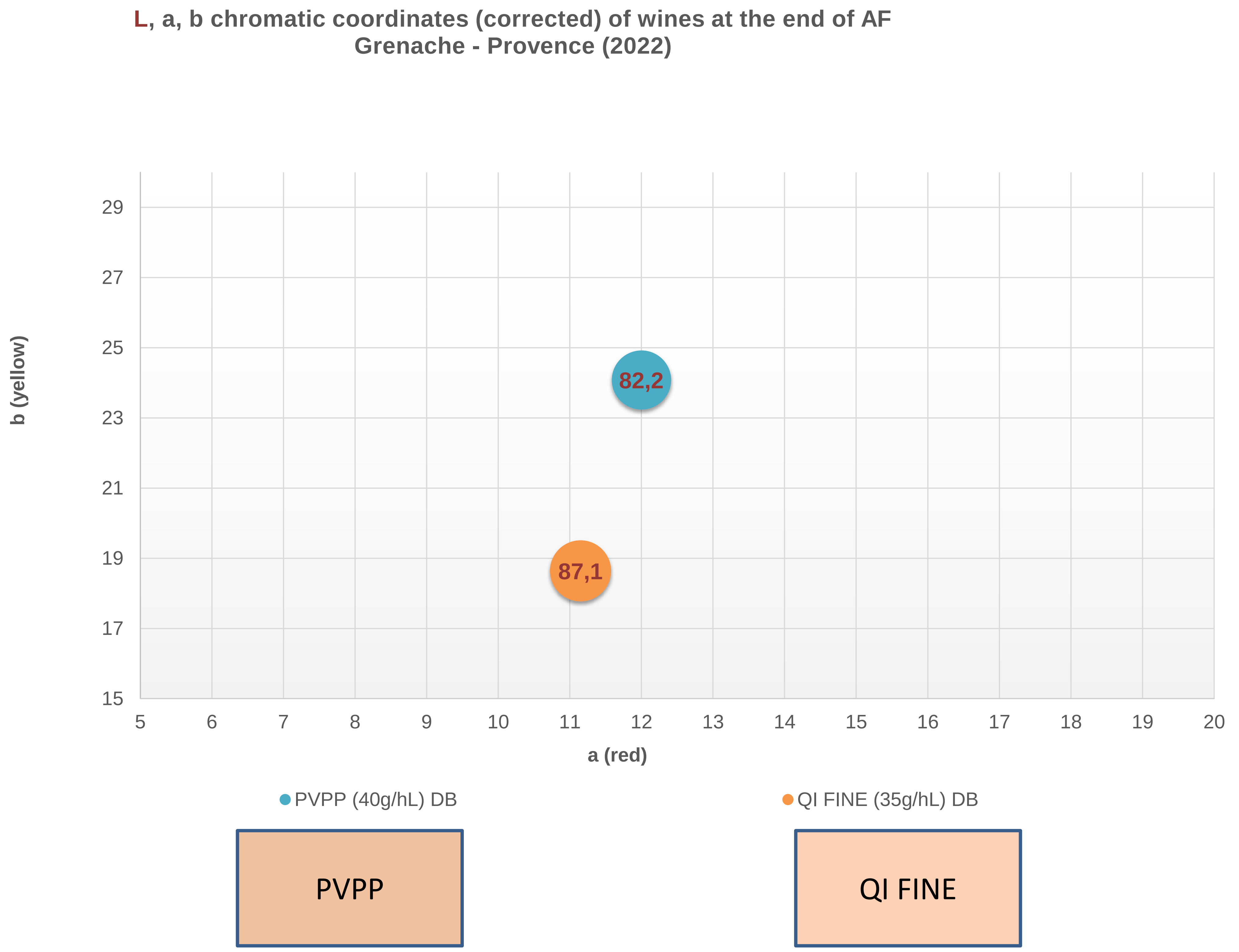
05 | Oenological chitosan
QI FINE – Fining agent for must and wines

Composition
Chitosan, pea proteins, tartaric acid

Impact
Decrease in bitterness and astringency, protection of the aromas, colour enhancement

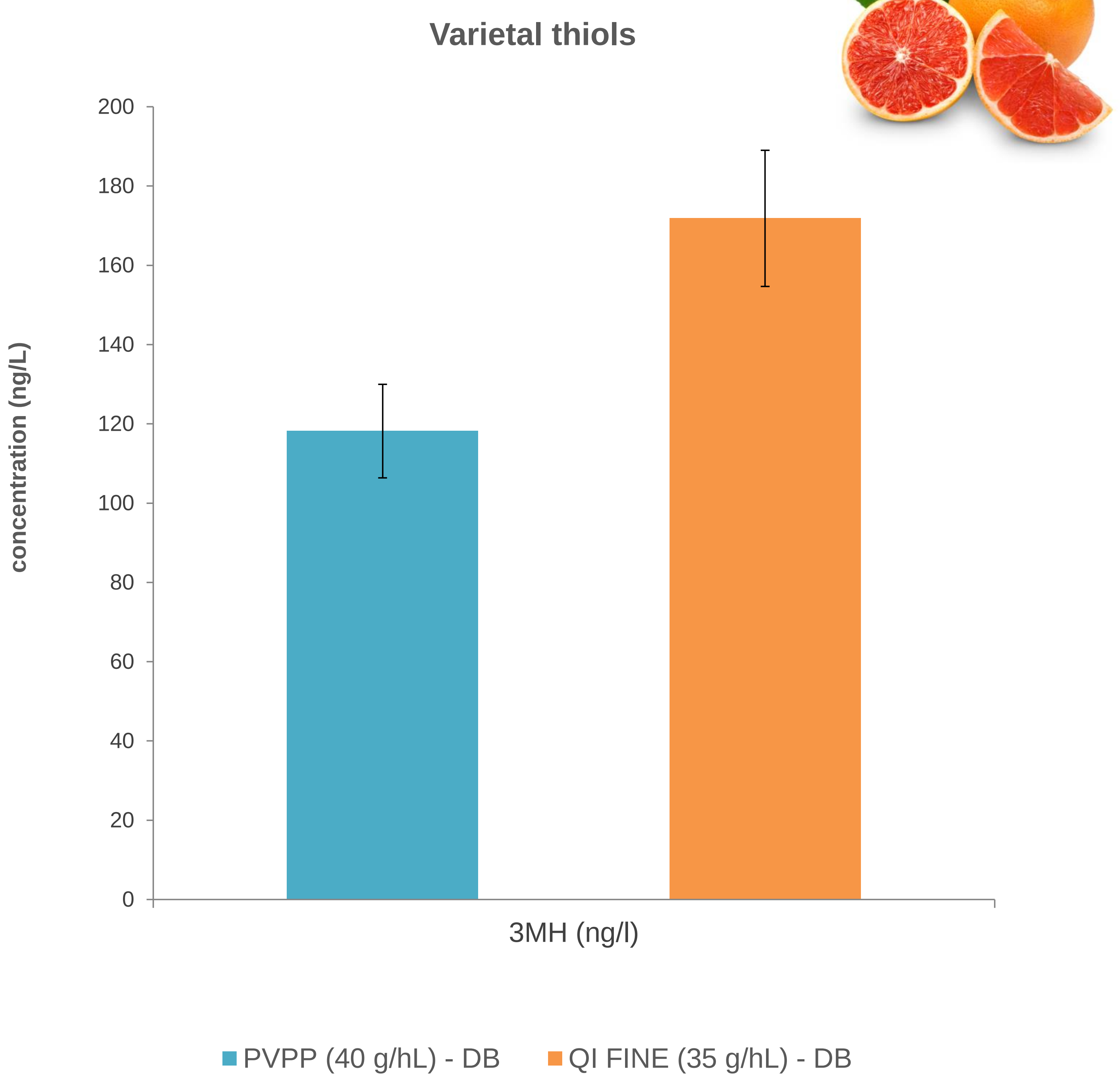
Available in powder and liquid formulation concentrated at 10%

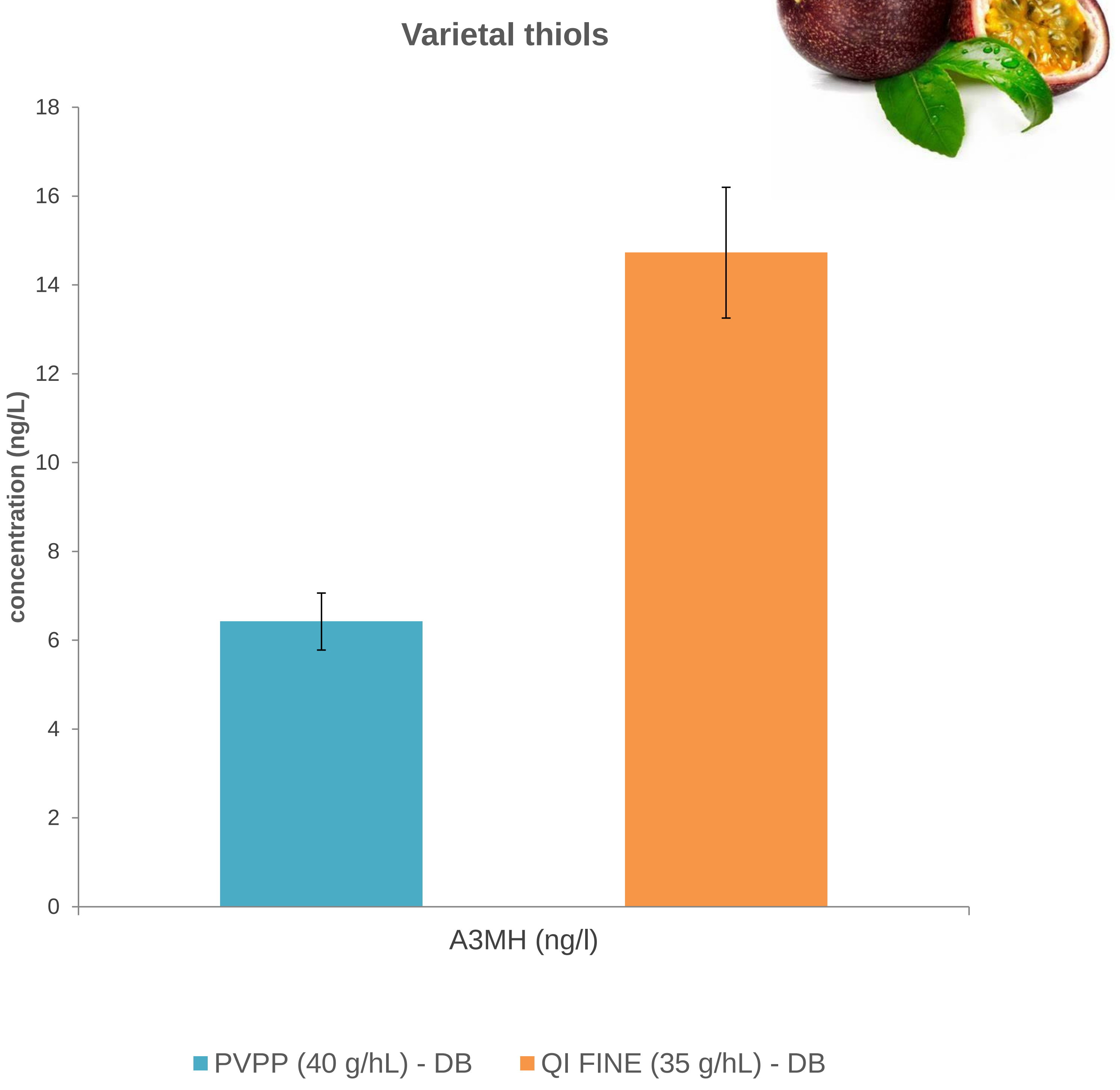
05 | Oenological chitosan
QI FINE – Trials in Provence - Color



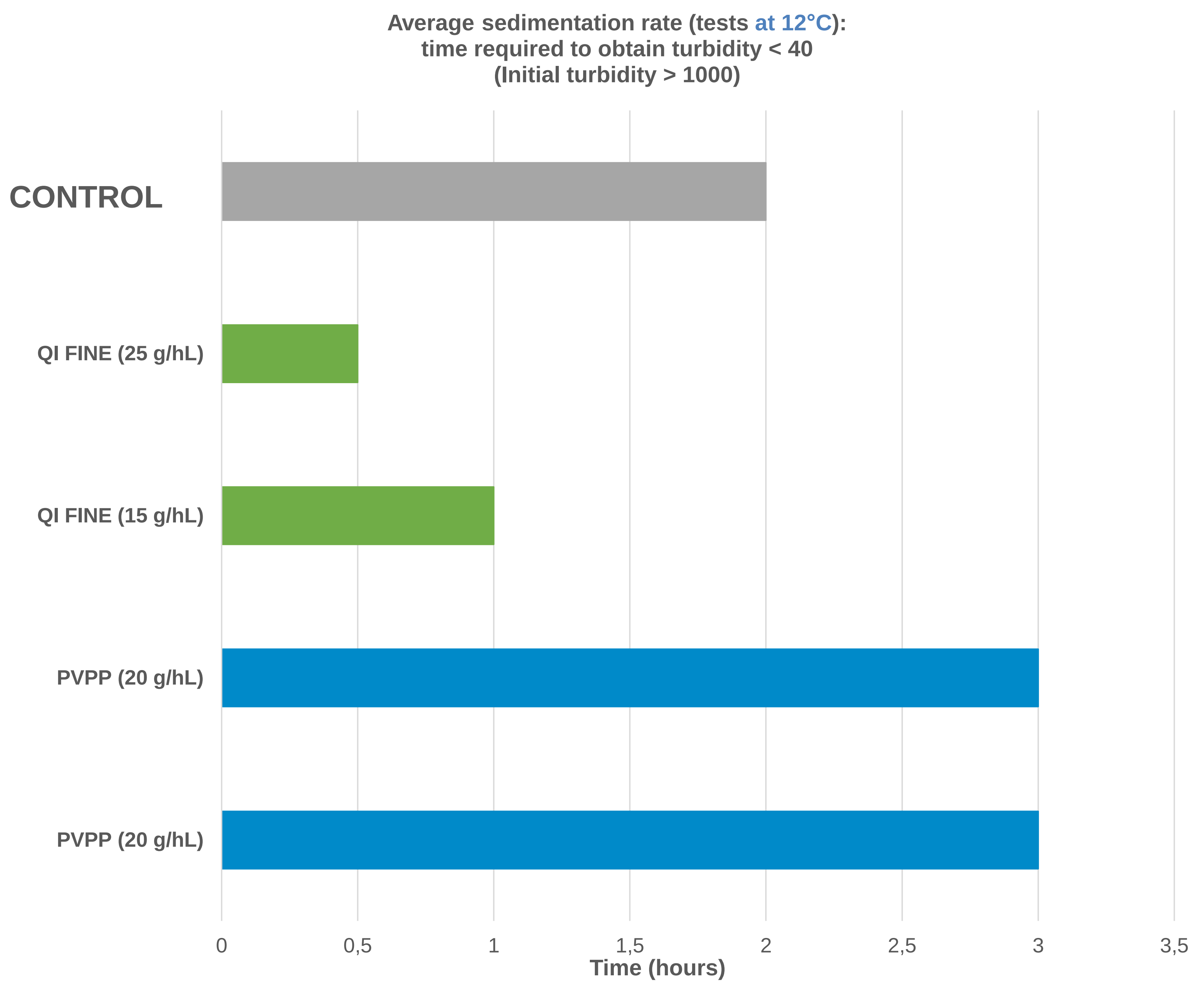
- Greater decrease in parameters a (red) and b (yellow) for the Qi FINE ™ treated model
- Clearer wine after treatment with Qi FINE ™

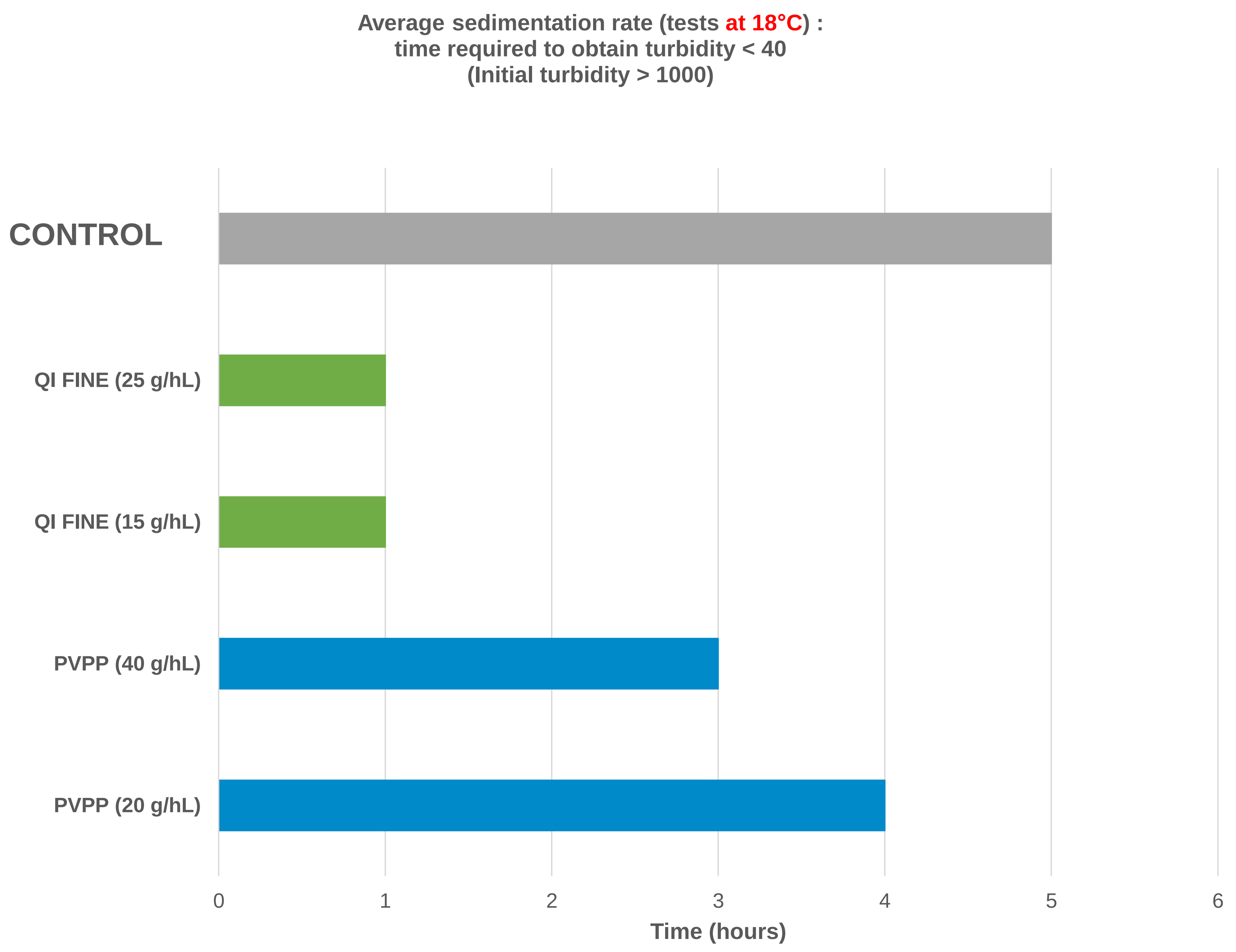
05 | Oenological chitosan
QI FINE – Trials in Provence - aroma





05 | Oenological chitosan
QI FINE – Trials in Provence – Sedimentation Kinetics





05 | Oenological chitosan
QI FINE vs PVPP

- + Air resistance
- + Yellow tint reduction
- + Thiol aromas (free radical scavenger role of chitosan)
- + Sedimentation kinetics
- = Final turbidity
- = Lees rate
- = Price (at recommended doses)

05 | Oenological chitosan
QI UP - Flottation

Composition
Chitosan, Tartaric acid

Impact
Fast clarification,
protection of the aromas,
colour enhancement

Available in powder and liquid
formulation concentrated at 10%

05 | Oenological chitosan
QI NOOX - Curative fining solution

Composition
Pea protein, Chitosan, Bentonite

Usable in Bio (1)

Indicative dosage:
10 to 50 g/hL

Impact
Colour improvement, freshness

Available in powder formulation

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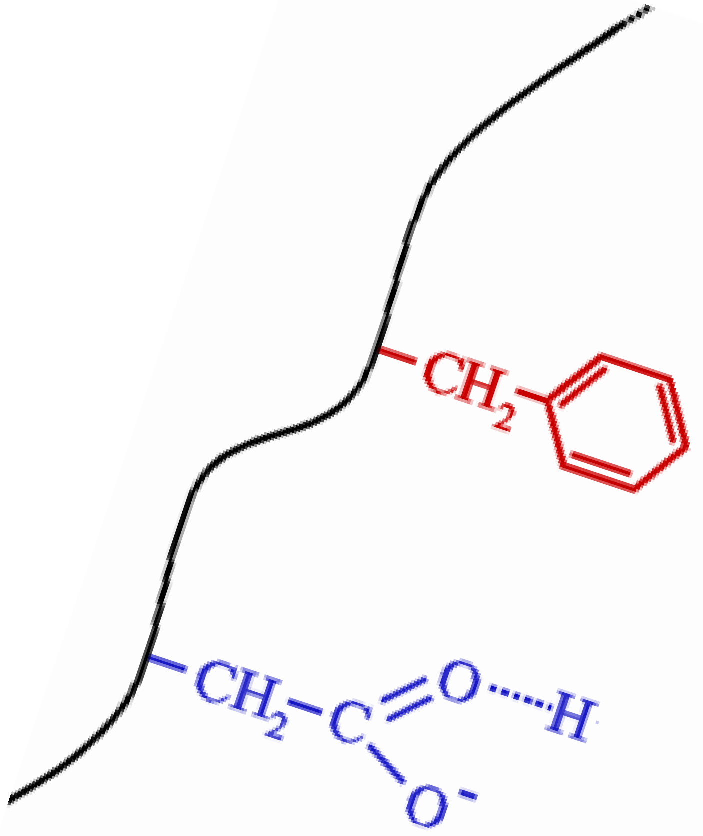
Stabilisation of aromas before bottling

06 | ULTIMA JUMP
When and in what form should mannoproteins be added?



Pre-fermentation phase

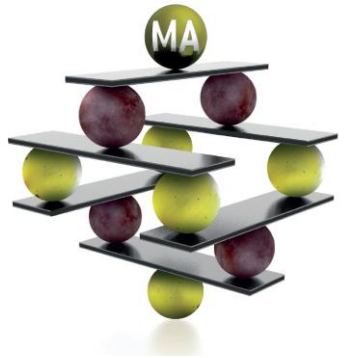
Inactivated yeast rich in mannoproteins

Before bottling

Formulation of yeast mannoproteins

06 | ULTIMA JUMP
Use before bottling

ULTIMA JUMP



- Mannoprotein-based formulation
- 100% soluble
- In granulated form
- Interacts with the aromas of white and rosé wines:
  - Preserving aromatic freshness
  - Flavour stabilisation
  - Aromatic clarity (less oxidation)

06 | ULTIMA JUMP
Example of sensory analysis results