Estaan: As an Alternative to SO_2 in Winemaking

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Outline

1. Wine preservation through time
2. SO_2 in winemaking
3. Drivers for reducing SO_2 today
4. My research: plant-based tannins
5. Conclusions & future perspectives

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Wine preservation

Wine has always been subject to conservation practices; since *otherwise its deterioration is certain and happens quickly!!*

Without protection, oxidation and microbial spoilage mask terroir expression

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A Historical Overview

Residue analysis of Middle Bronze Age clay jars (1900-1600 BC) revealed many products used as preservatives and flavorings (Koh et al., 2014)

www.nationalgeographic.com

Honey

Cedar oil

Cyperus

Juniper

Mint

Myrtle

Cinnamon

Each jar had a varied ratio of ingredients

Storax resin

Terebinth resin

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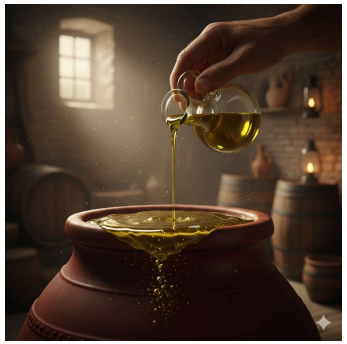
Preservation in Ancient Times

Greco-Romans used several procedures to stabilize and preserve wine



Olive oil ≈ protection

jerez-xeres-sherry.blogspot.com

Biological protection

https://eurogypsum.org/

Gypsum ≈ Acidity

Heliasterion ≈ Pasteurization

shedevrum.ai

Salts ≈ biological stability

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Modern SO_2 Levels and Consumer Perception

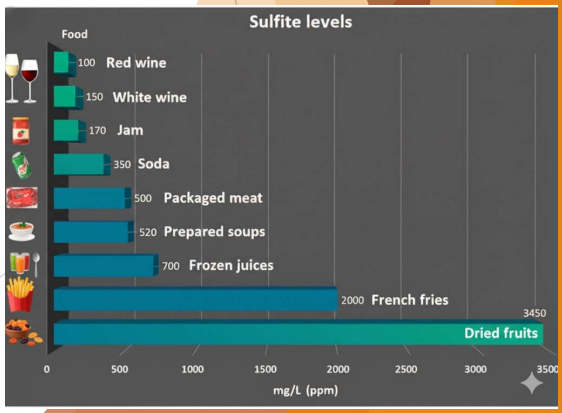
Consumers only began to widely question the use of sulfites after 2005, when EC Regulation 1991/2004 required wine labels to state *"Contains sulfites"*

Organization / country	Type of wine / Residual sugar	Authorized limit (mg/L)
EU	White and rose < 5 g/L	200
	Red < 5 g/L	150
	White and rose ≥ 5 g/L	250
	Red ≥ 5 g/L	200
	Special sweet wines	300 - 400
EU	Organic White and rose > 2g/L - < 5 g/L	170
	Organic Red > 2g/L - < 5 g/L	120
	Organic White and rose ≥ 5g/L	220
	Organic Red ≥ 5g/L	170
	Organic Special sweet wines	270 - 370
Codex Alimentarius		350 mg/kg
OIV	White and rose < 4 g/L	200
	Red < 4 g/L	150
	White and rose ≥ 4 g/L	300
	Red ≥ 4 g/L	300
	Special sweet wines	400

Over 10.000 samples:

- White wines ≈ 132,8 mg/L
- Red wines ≈ 96,79 mg/L

(Vinetut, 2025)



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SO_2 in winemaking

Properties	Advantages	Disadvantages
Antimicrobial	- **Control of undesired yeast and bacteria** populations	- **pH-dependent**
		- Greater resistance of acetic bacteria
Antioxidant	- **Reacts with H_2O_2** inhibiting the formation of aldehydes	- pH and **grape health dependent**
	- **Restoring quinones**, to their phenolic form **(Protects from browning)**	
Antioxidasic	- **Inactivates oxidase enzymes**	- pH and grape health dependent
Extractant	- **Extraction** of minerals, organic acids and phenolic compounds	- Crushing grapes immediately removes SO_2.
Flocculant	- **Clarifying action**	
	- **Promotes precipitation** of lees	
Sensory	- **Improves flavor and aroma** (combined with acetaldehyde)	- **High doses neutralize varietal aromas** and produce **unpleasant notes of reduction** (H_2S).
	- **Improves** the flavor **of wines made with rotten grapes**	- Formation of mercaptans (cauliflower, mushroom)
		- **Burning aftertaste**
Technological	- **Low cost** (compared to others additives)	- **Corrosion of cement and metal tanks**
		- **Difficulties in handling and dosing**
		- Depletion of oak aromatic compounds

Drivers for reducing SO_2

F. Salinas (2025), created with Nano Banana

Tannins as SO_2 alternative

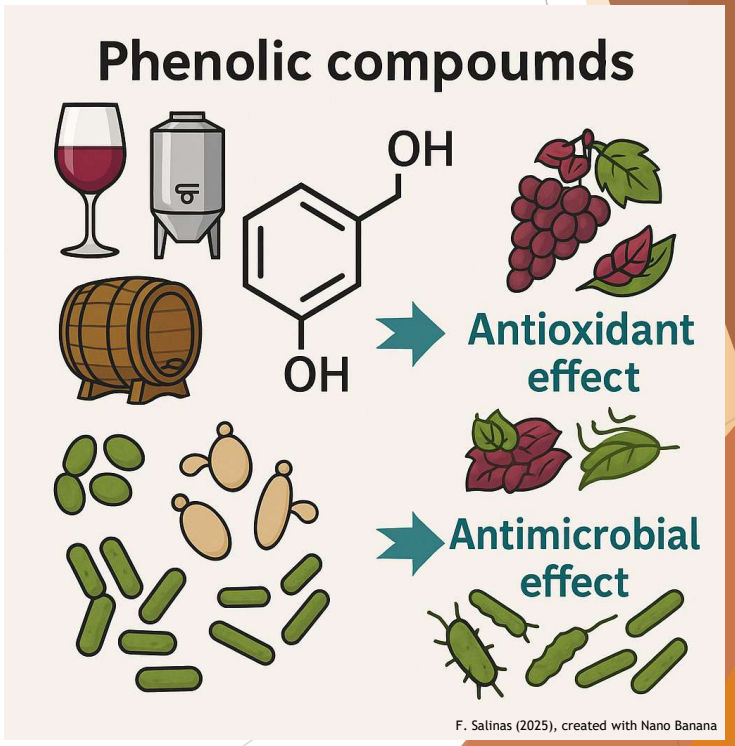
Tannins have been shown to be effective as *antioxidants* (Yıldırım, 2013)**, and have *antimicrobial properties*** (Silva et al., 2018)**.**

Their antioxidant activity is attributed to:

- **donating hydrogen atoms to free radicals,**
- **scavenging other reactive species such as OH**
- **or by binding transition metal ions**

The antimicrobial activity is attributed to:

- **Alteration of cell components' structures**
- **Protein denaturation**
- **Generation of oxidative stress**

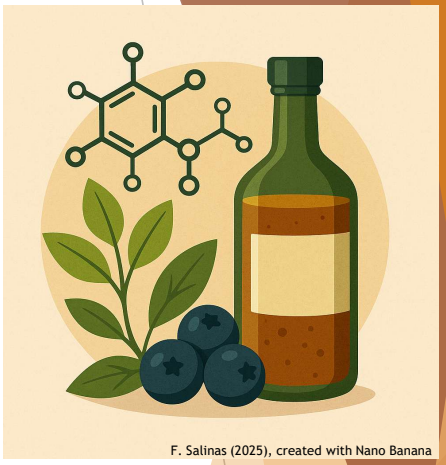


Oenological tannins "Estaan"

Trade name: ESTAAN PLANT BASED SULPHITE REPLACER

Estaan is a standardized matrix of tannins from plant extracts

Produced by: Biolethics Europe, from the Netherlands



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Plants used for the extraction: *Rosmarinus officinalis, Vaccinium myrtillus, Morus alba, Melissa officinalis, Ananas comosus, Mangifera indica, Punica granatum, Prunus domestica, Lycium chinense fructus, and Ruta graveolens.*

ESTAAN fully complies with the International Oenological Codex of the OIV regulation OIV-OENO 624-2022

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Food Safety certificates

- ✓ **Certificate of analysis Eurofins**
- ✓ **AMES test Eurofins** (Mutagenicity test)
- ✓ **Health Certificate Meron** (for human consumption)
- ✓ **Analysis of IEZ**

eurofins | Food Safety Solutions

SGS

C&R/AFL Laboratory
Malledijk 18
P.O.Box 200
3200 AE Spijkenisse
Dir Tel +31 (0)88 214 45 00
Email: nl.afl.lab@sgs.com

VEGAN VERIFIED
eurofins | Chem-MAP

This is to certify that

BIOLETHICS EUROPE

Has been awarded Vegan Verification for the products listed in Table One with reference to:

Contract number: RCR21-165
Company ID: 5462

We find BIO-ESTAAN, absolutely aligned with the H2020 framework priorities, as well as a strategic and innovative solution in the wine Industry short-term improvement, based on a better food security and health for European citizens.

Sincerely,

Rustemeyer

Prof. Dr. Dr. Thomas Rustemeyer
Department of Dermatology and Allergology
Asterdam university medical centers
Amsterdam
The Netherlands

- Estaan is GMO free
- No toxic compounds
- Allergen free certificate
- Microbial and pesticide / herbicide contamination checked
- Estaan contains no protein

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Test vinifications

- Trials at Geisenheim University (since 2019)
- Larger-scale trials in Germany (2023)
- Other trials carried out in other countries (2019-now)

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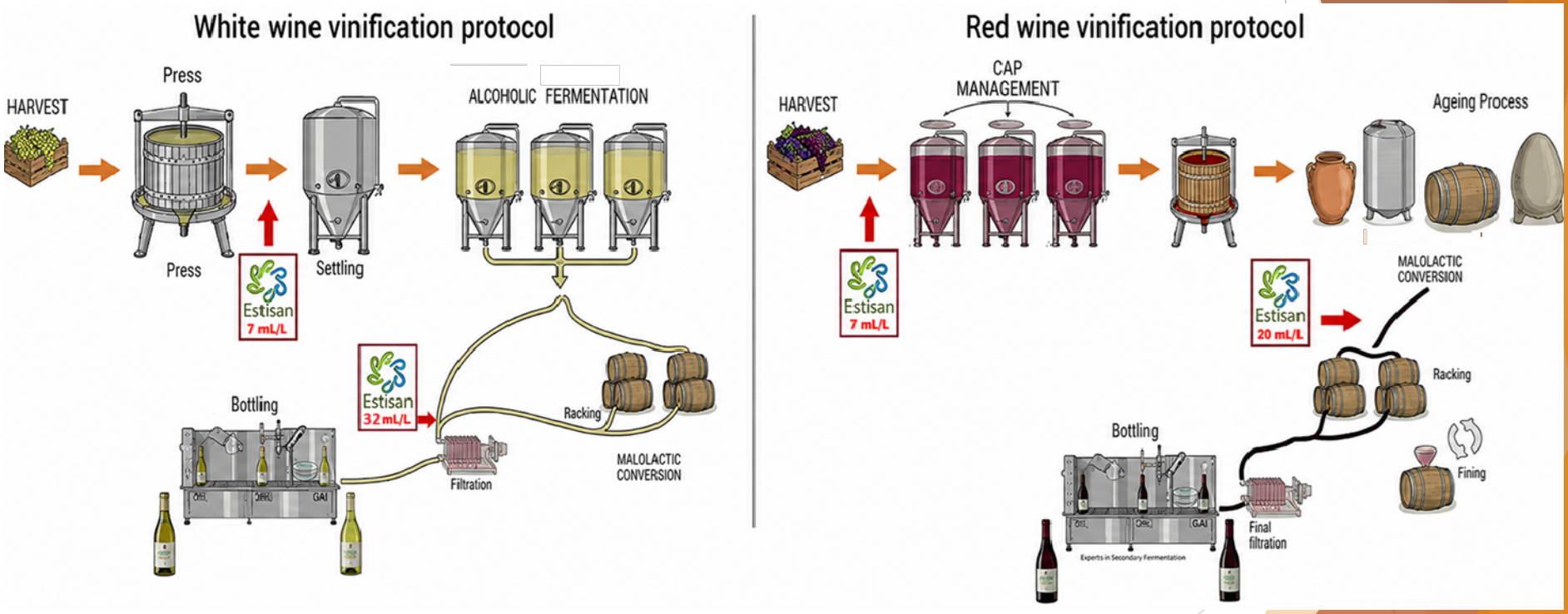
Winemaking protocols



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Key Results

Parameter group	Effect of Estaan vs SO_2 / Control	Take-home message
N-OPA	**No significant differences**; no N depletion	**Safe for yeast nutrition**
FTIR parameters	Within normal range; no systematic shifts	**Technologically neutral** on key parameters
AF kinetics / residual sugar	Complete AF in all treatments; no stuck ferments	**Fermentation is not** negatively **impacted**
Lees formation	**Consistently higher lees volume with Estaan**	Potential advantage for clarification
Protein stability	Similar or improved stability; no extra bentonite demand	No negative impact on protein stability
Acetaldehyde	**Consistently lower acetaldehyde in Estaan wines**	Lower carbonyl stress and potential SO_2 demand
Colour (rosé, red)	Preserves or enhances colour; mitigates browning	**Positive impact on visual quality**
Colour (white)	**Yellow color of greatest intensity at 420 nm**	Impact on color and visual perception

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Oxidation assays

Control:

Enzymatic oxidations:
Laccase activity test

Non-enzymatic oxidations:
Oxygen consumption ratios

Antioxidasic evaluation

Aim: Estimate the ability of Estaan to inhibit laccase activity produced by *Botrytis cinerea*.

Antioxidant evaluation

Aim: To measure the kinetics of oxygen consumption by Estaan in a model wine solution.

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Antioxidasic Evaluation

Riesling grapes affected by *Botrytis* were selected, and laccase activity was evaluated using the *syringaldazine* method.

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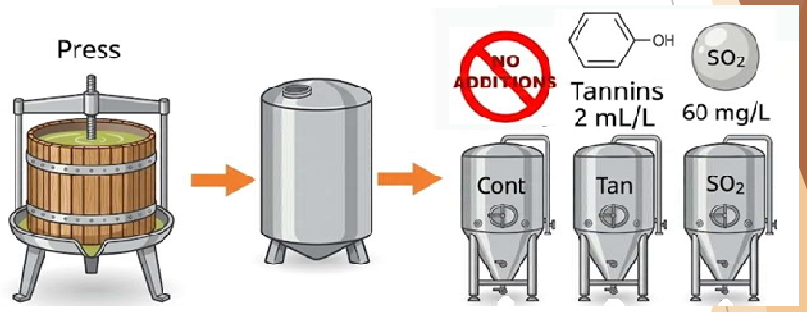
Riesling grapes showed 50–70% *Botrytis* incidence



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Density 20/20	Total extract g/L	Residual sugar g/L	pH	Total acid. g/L	Tartaric acid g/L	Volatile acid g/L
1,0846	220,4	196,8	2,8	11,5	7,8	0,5

Malic acid g/L	Ethanol g/L	Gluconic acid g/L	Glycerin g/L	Free SO2 mg/L	Total SO2 mg/L	Total phenols mg/L
5,3	0,1	1,5	1,4	4	25	249

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Infection levels corroborated by laccase activity test, gluconic acid and glycine levels

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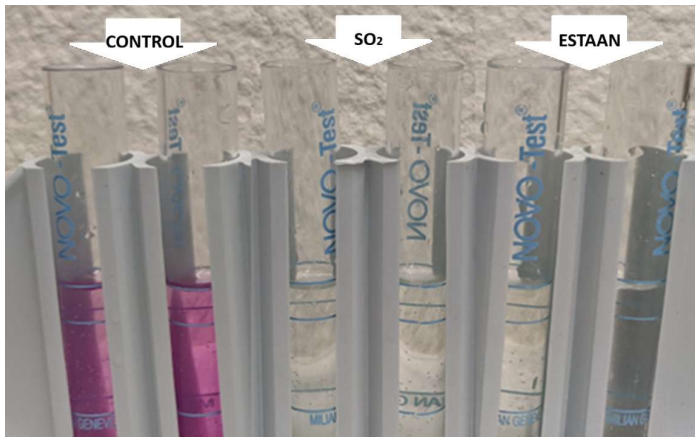
Antioxidasic Evaluation

Results

For laccase activity, significant differences were recorded between the control and both SO_2 and Estaan treatments, while no significant differences were observed between SO_2 and Estaan.



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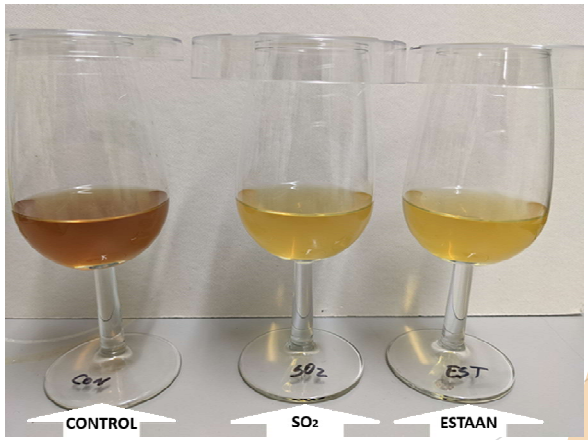
Botrytest results for laccase activity, at the time of pressing (0 hour).



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Treated musts, after 24 hours

Antioxidant evaluation

Component	Target concentration
Ethanol	12 % v/v
Tartaric acid	4 g/L
pH adjustment (NaOH)	pH = 3.5
Iron	3 mg/L Fe
Copper	0.3 mg/L Cu

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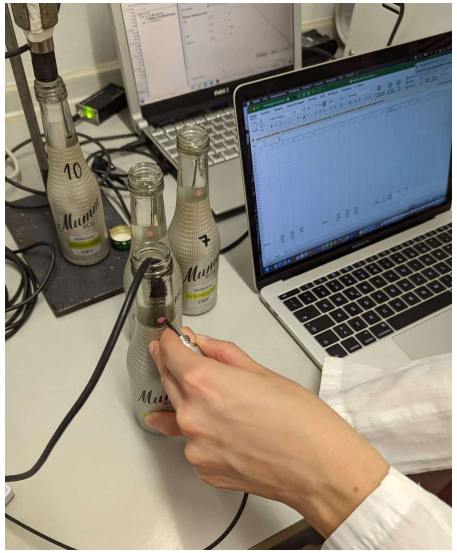
The model wine solution was saturated with 8 mg/L of oxygen. Treatments were maintained at a constant temperature of 20°C.



F. Salinas (2023)

F. Salinas (2023)

Clear glass bottles fitted with a sensor pill were used to measure dissolved oxygen. All solutions were saturated with oxygen and kept at a constant temperature of 20°C.

Antioxidant evaluation

Results

Estaan tannins at 2 mL/L consume all oxygen, showing greater antioxidant power and faster oxygen consumption than SO_2 treatments.

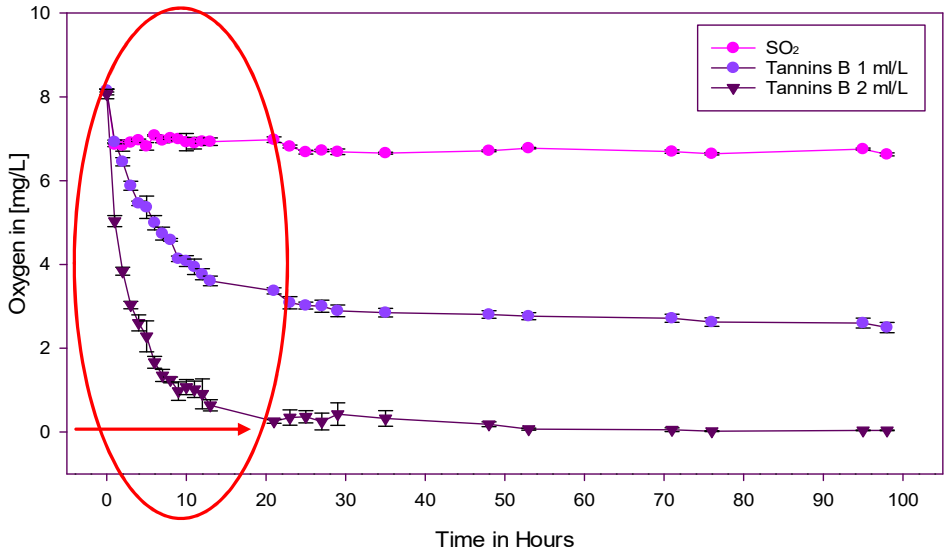


Figure 6. Kinetics of oxygen consumption rate for SO_2 and Tannins, at doses of 1 ml/L and 2 ml/L.

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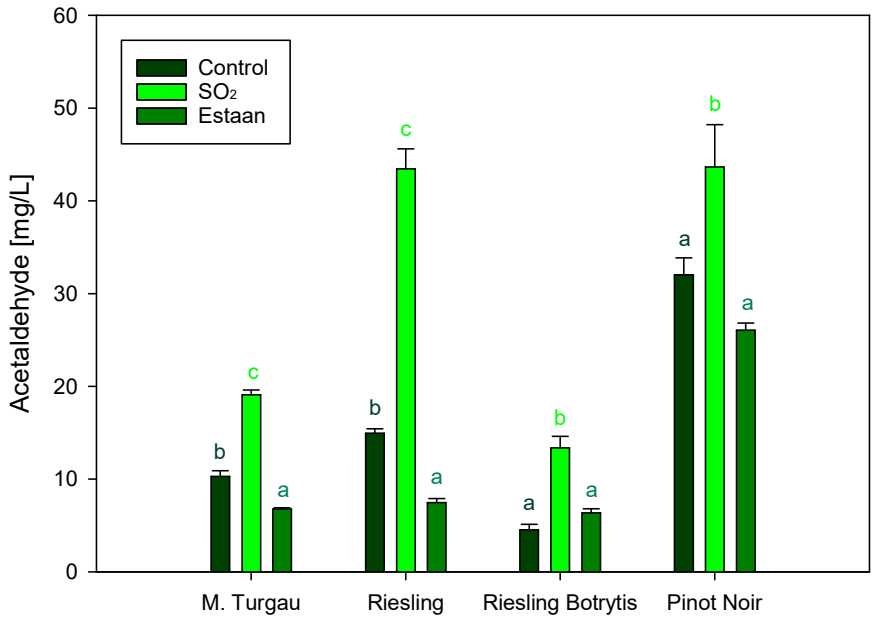
Acetaldehyde as an Oxidation Marker



Lower levels of acetaldehyde recorded in treatments with Estaan

Microbiological Assays

Control of *O. oeni*

Monitoring malic and lactic acid levels in inoculated wines

Control of *Brettanomyces*

Growth monitoring in Petri dishes

Oenococcus oeni

Aim: To assess the ability of Estaan to control malolactic fermentation.

Brettanomyces spp.

Aim: To assess the ability of Estaan to control *Brettanomyces,* associated with contaminated wood.

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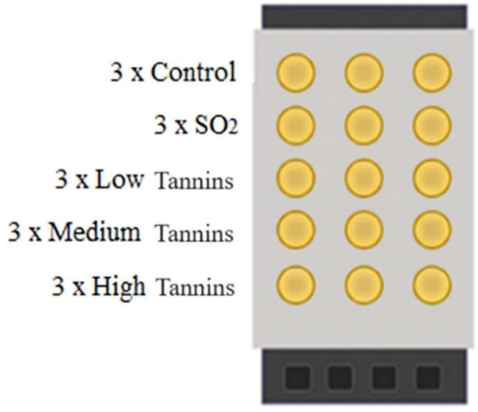
Oenococcus oeni test

5 Bottles

1 Control (No additions)
1 Control (50 mg/L of SO_2)
1 Low Tannins (1 mL/L)
1 Medium Tannins (2 mL/L)
1 High Tannins (3 mL/L)

pH	Malic acid g/L	Lactic acid g/L	Total SO_2 mg/L	Free SO_2 mg/L	Total phenols mg/L
3,2 ± 0,0	4,7 ± 0,0	0,3 ± 0,0	11,0 ± 0,1	2,0 ± 0,1	117,0 ± 0,0

Monitoring malic acid levels / 7 weeks
enzymatic kit



Inoculation

250 mL of wine / cylinder

Inoculations with *O. oeni*
Bi-Start Vitale SK11 (Erbslöh)

10^6 CFU/mL

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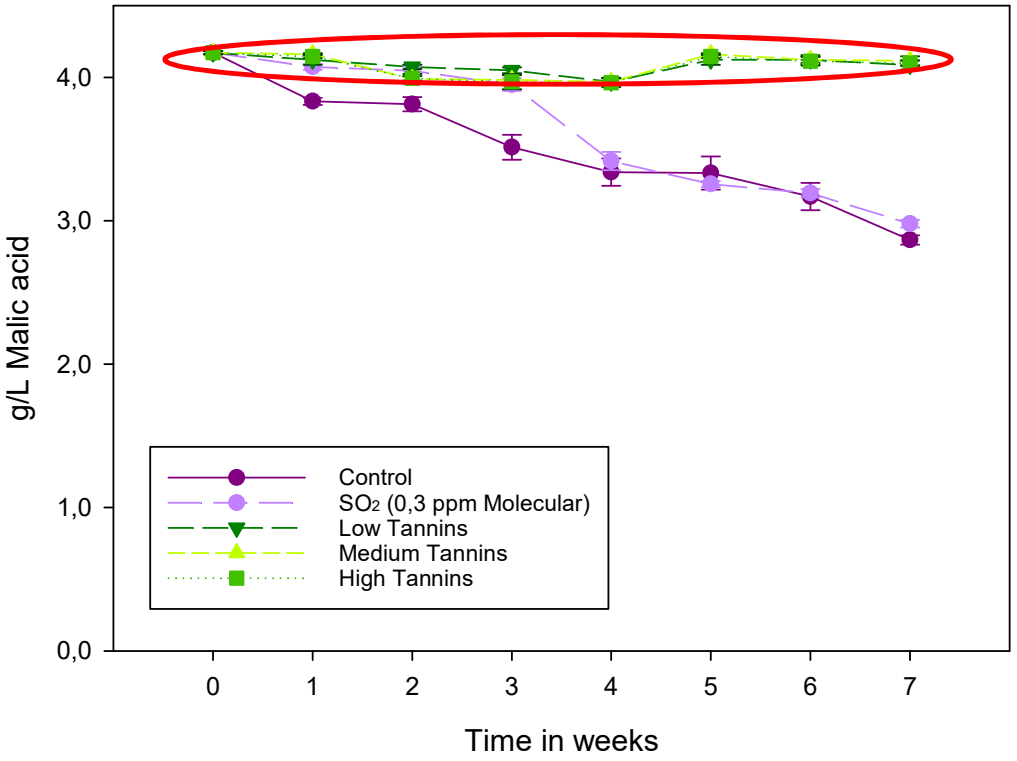
Effect of Estaan on *MLF*

Results



- All phenolic treatments maintained malic acid levels in the seventh week
- Control treatment: MLF from the first week
- Treatment with SO_2: MLF from the third week

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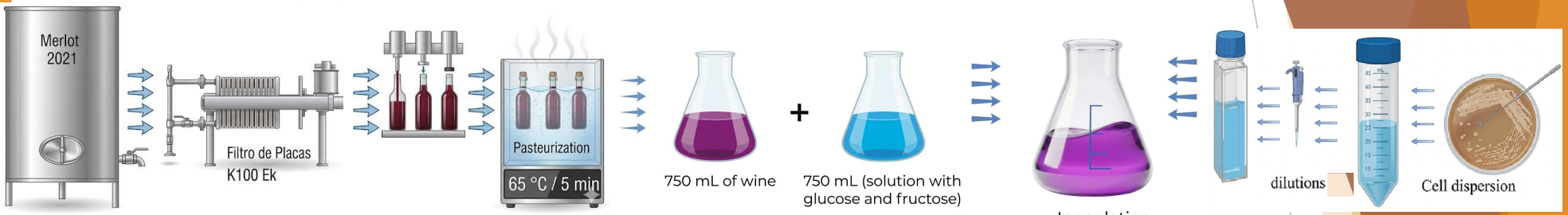
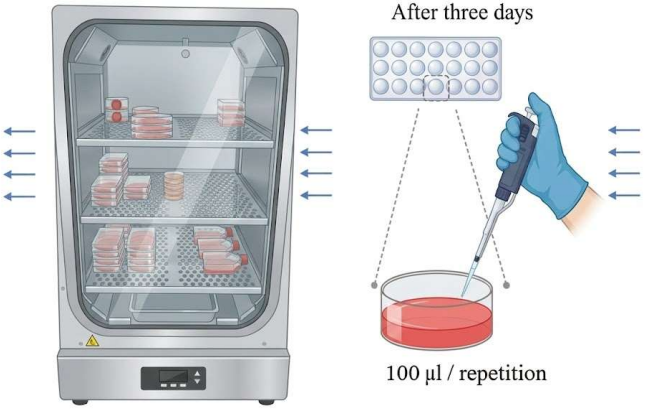
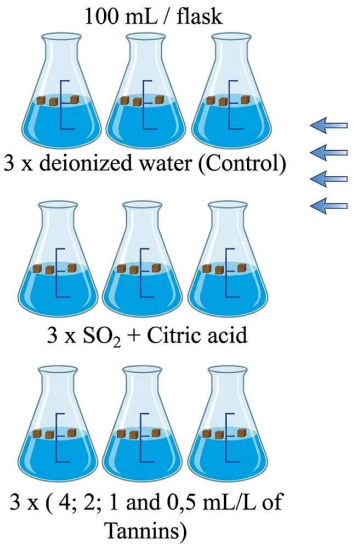
Brettanomyces test

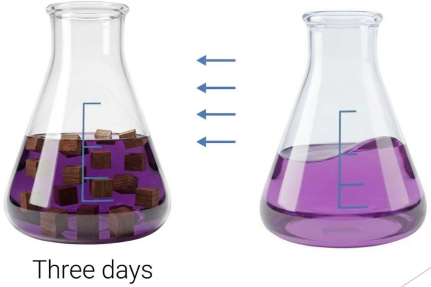


Brettanomyces yeast, 21B37 strain.

Plate Count Method







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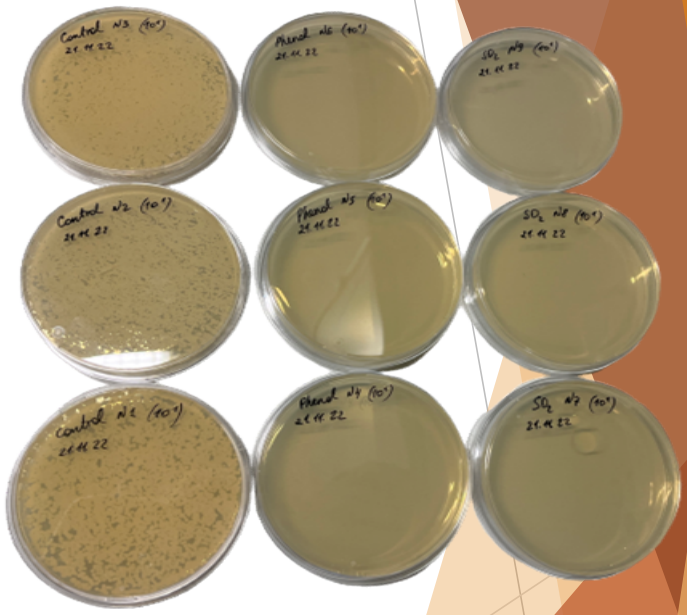
Brettanomyces Inhibition

Results

Composition of the Solution	Yeast Amount (CFU/mL)	Neutralized Yeast (%) Compared to Control	Logarithmic reduction
Distilled water (control)	34.667 ± 3.786	---	---
SO_2 + citric acid	0 ± 0	100,00	---
Tannins 4 ml/L	0 ± 0	100,00	---
Tannins 2 ml/L	1.190 ± 85	96,57	1,46
Tannins 1 ml/L	7.550 ± 3.465	78,22	0,66
Tannins 0,5 ml/L	16.100 ± 1.414	53,56	0,33

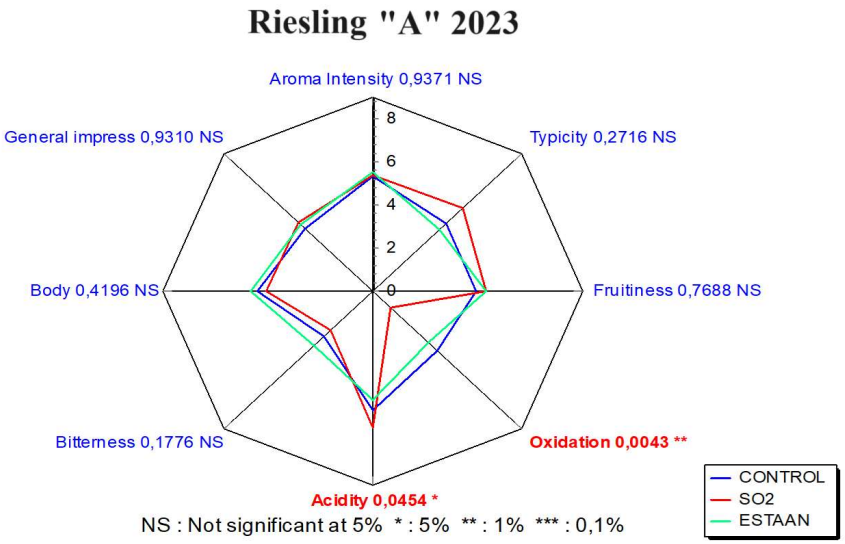
- A clear inhibition of the development of *Brett.* is observed, with different percentages of inhibition according to the doses tested.

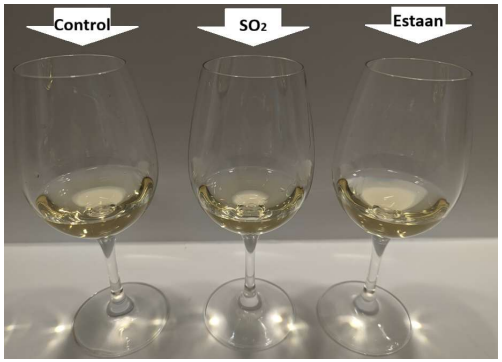
- The tannins, even at the lowest doses tested, exceed 50% inhibition.



Results of the Three-Day Exposure of Oak Chips and *Brett.* Yeast to the Working Solutions (Control, Tannins, and SO_2)

Sensory Analysis





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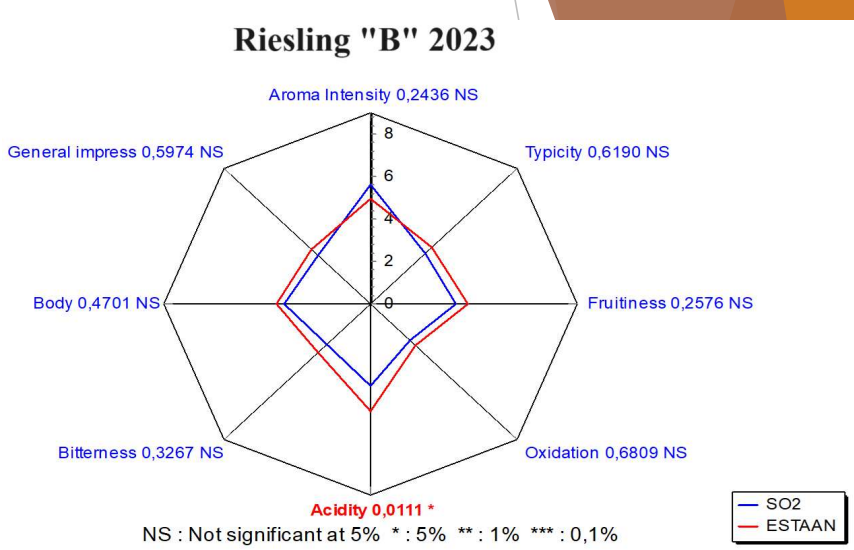

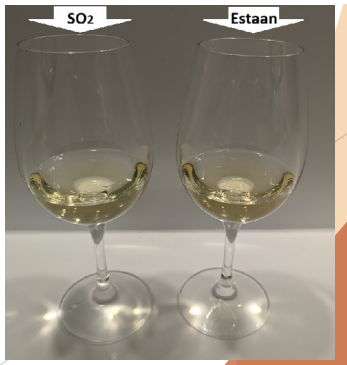

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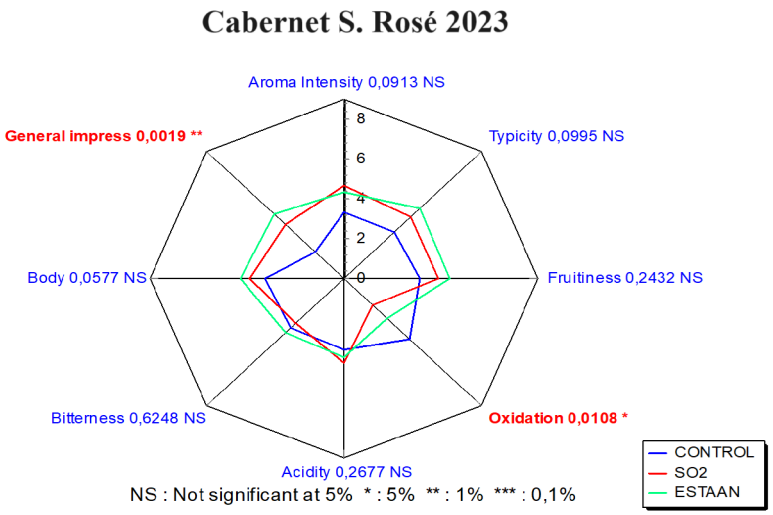

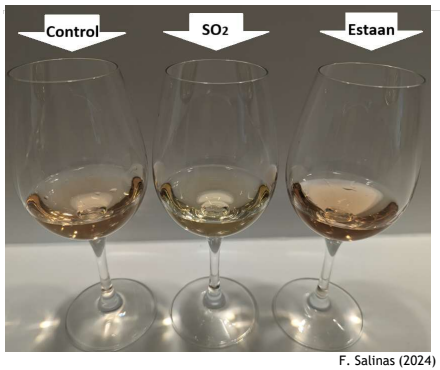

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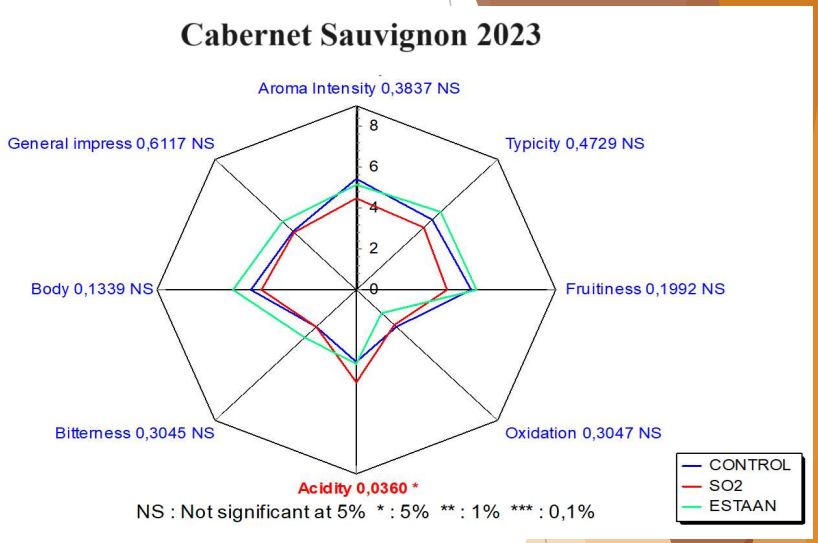

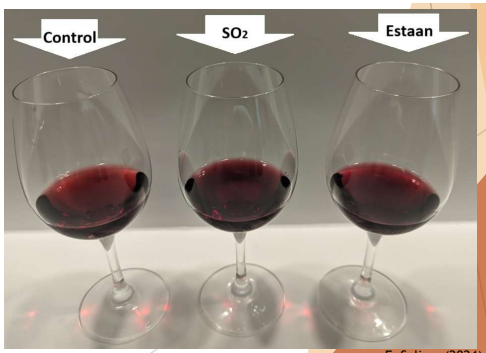

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Conclusions

Wine matrix: Estaan maintains **oenological parameters** within normal ranges, **without alteration of fermentative kinetics**.

Antioxidasic & antioxidant evaluation: Estaan showed **strong antioxidasic activity**, reducing laccase activity in Botrytis-affected musts, and provided **relevant antioxidant protection**, with fast oxygen consumption.

Microbiological evaluation: Estaan also showed **antimicrobial effects** against *Oenococcus oeni* and *Brettanomyces*

Sensory evaluation: Estaan performs **best in rosé, red and Botrytis-affected wines**, while delicate **white wines** remain the most sensitive and **require fine-tuned protocols**.