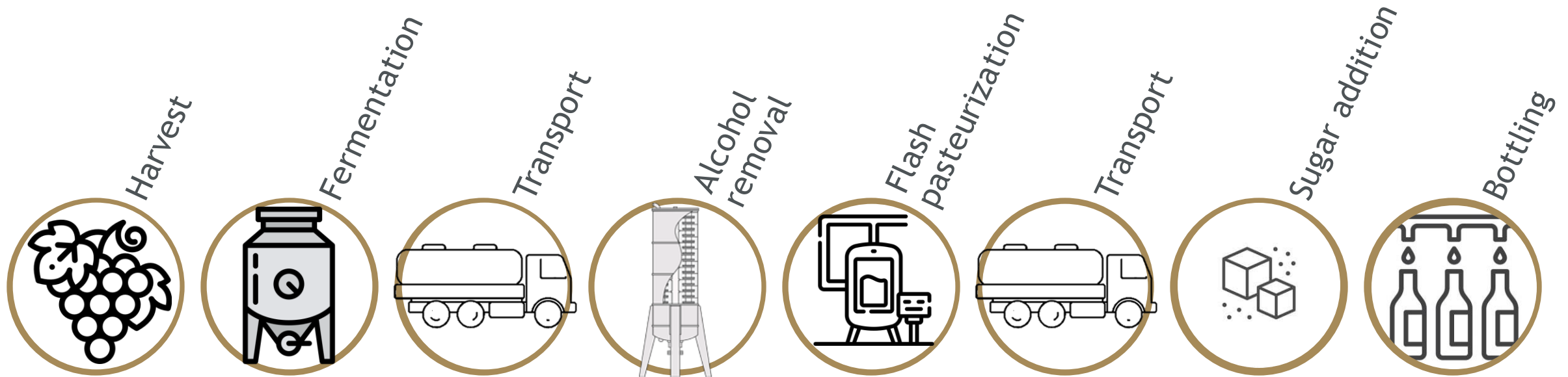


Zygosaccharomyces: Is there a threat looming in alcohol free wines?

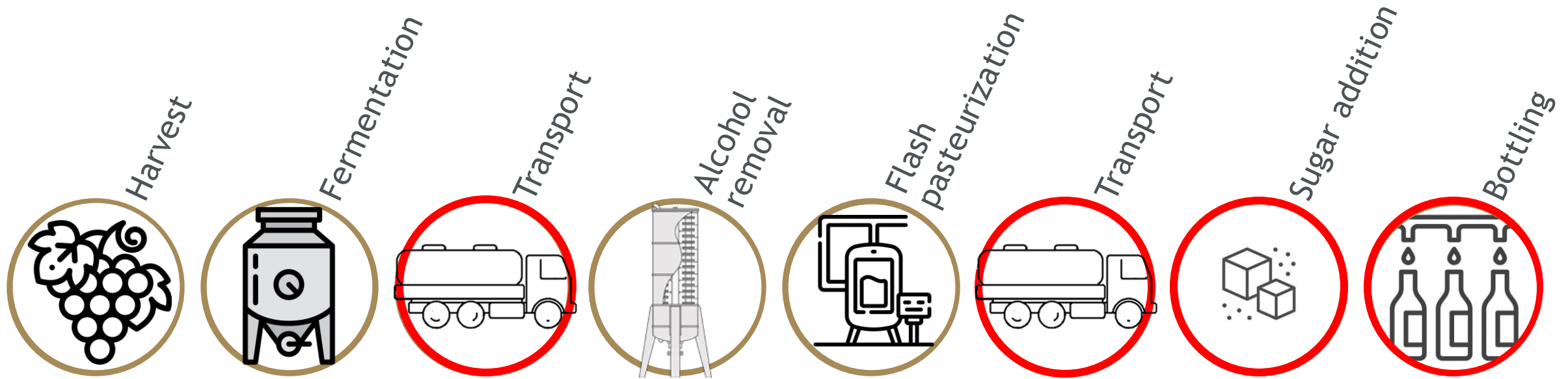
Student: Luke Stephen Pulford
Supervisor: Prof. Maret Du Toit

Low- and no-alcohol wine production



- Transport to and from alcohol dealcoholisation facility is a common practice for producers without in-house equipment.
- Sugar addition in the form of concentrated grape musts or crystallized grape sugars is important to achieve a sensorily balanced low- or no-alcohol wine.

Low- and no-alcohol wine production risks



- High risk practices are those which have the potential of introducing unwanted microbes into the product.

Preservative additives

The International Organization of Vine and Wine stipulate guidelines for preservative agents which are permitted in wine.

Permissible additives

Sulphur dioxide
(SO₂)

- Enters cells and disrupts cellular function, reduces respiration and acidification of intracellular environment.

Sorbic acid

- Inhibition of certain enzymes, inhibition of respiratory growth of yeast.

Dimethyl
dicarbonate (DMDC)
Trade name Velcorin

- Reacts with proteins, amino acids and nucleic acids.

Chitosan (from
fungal origin)

- Plasma membrane structure impairment, leakage of compounds from cell, disruption of protein synthesis, chelation with metal ions, combination of nonspecific, simultaneous interactions.

Non-permissible additives

Pimaricin (commonly
referred to as
Natamycin)

- Binds to ergosterol in the cell membrane irreversibly which results in an increase of permeability and leakage of ions and small peptides.

Benzoic acid

- Inhibition of growth and fermentation with potential reductions of ATP.

(Schimz & Holzer, 1979), (Maier *et al.*, 1986), (York & Vaughn, 1964), (Stratford *et al.*, 2020), (Henderson, 1973), (Solymosy *et al.*, 1971), (Raafat *et al.*, 2008), (Galván Márquez *et al.*, 2013), (Kong *et al.*, 2010), (Munn *et al.*, 1999), (Warth, 1991)

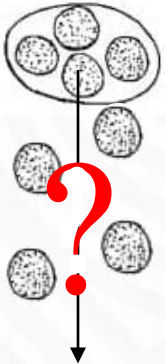
Physical preservation methods

Frequently used:

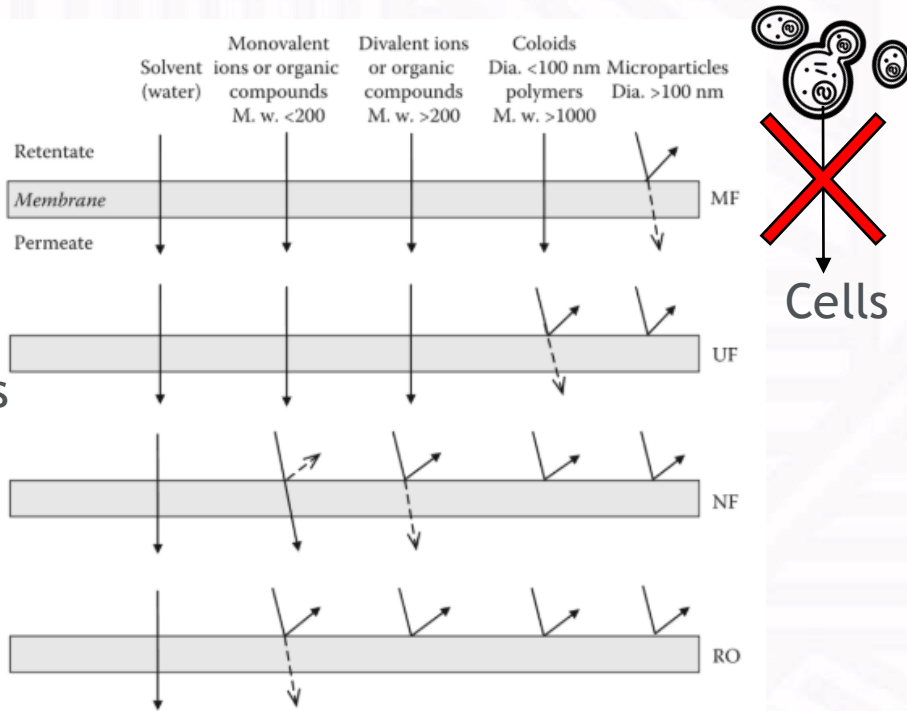


Sterile filtration

Filtration through a pore diameter $\leq 0.45 \mu\text{m}$



Ascospores



Commercial but emerging



Ultra-violet radiation (UV-C)

Effective antimicrobial action. Nucleic acids of organisms are rearranged preventing DNA replication and transcription. Is explored in industry.



Ultrasound (High Power Ultrasounds - HPU)

Production of free radicals which impair cell wall physical structure, causes cavitation which creates a shearing force disrupting cell structures.



High hydrostatic pressure (HHP)

High pressure for a short period of time reported to be highly effective at inactivating cells.



Pulsed electric field (PEF)

Short durations of electrical pulses that results in membrane electroporation which has been shown to increase permeability.

(Schlosser, 2013), (Bullé Rêgo *et al.*, 2022), (Mok *et al.*, 2006), (Tsong, 1991), (Ganeva *et al.*, 2014), (Wu *et al.*, 2012), (Gracin *et al.*, 2016), (van Wyk & Silva, 2017)

My research



In progress/Ongoing



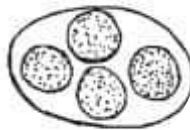
Analysis of spoiled low- and no-alcohol wines



Development of eSurvey to gain industry insight



Identification of spoilage yeast



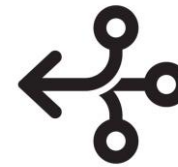
Assessment of *Zygosaccharomyces* sporulation and spore harvesting techniques



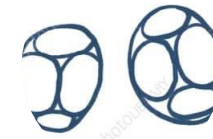
Analyse inhibition of isolated yeast by preservatives



Future



Analyse the effects of combined preserving agents to inhibit spoilage yeast



Analyse the germination of spores in low- and no-alcohol wine

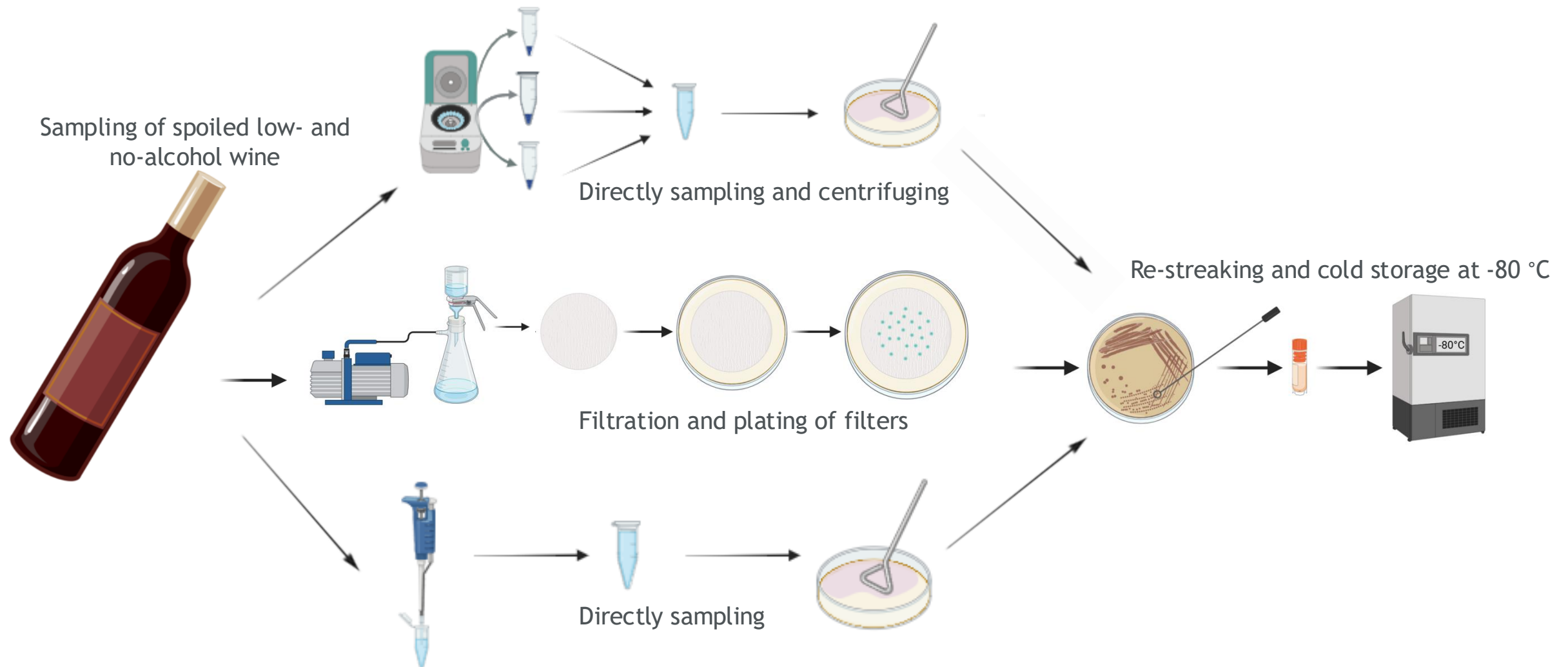


Analyse the efficacy of alternative physical preservation techniques (PEF, HHP, HPU)

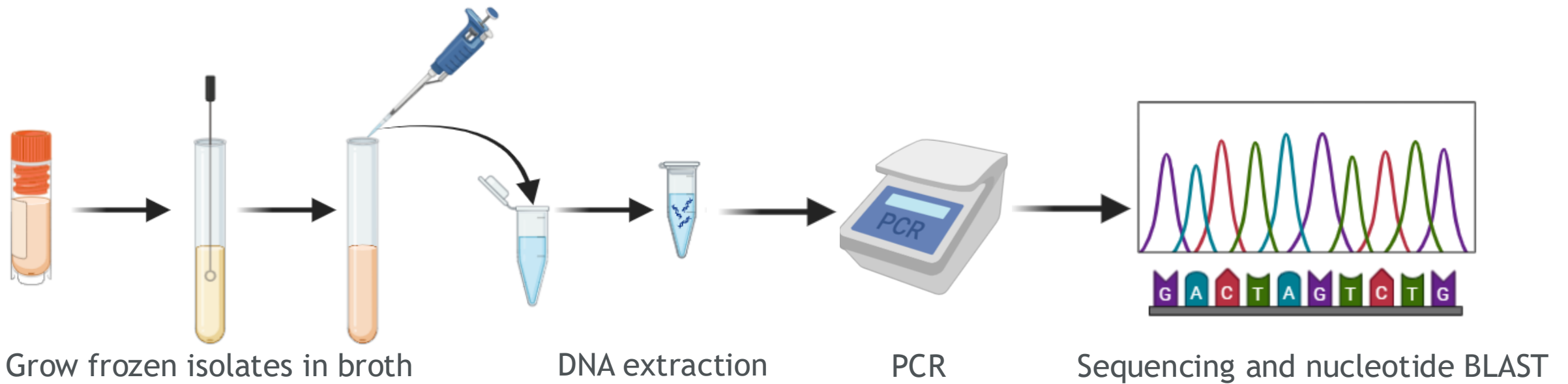


Analyse preservative inhibition of yeast spores

Analysis of Spoiled low- and no-alcohol wine



Identification of Yeast Isolates

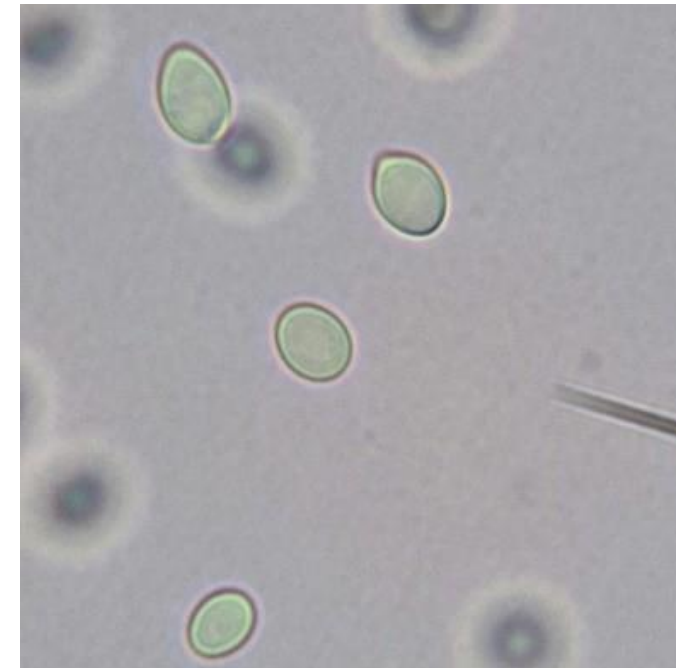


Identification of Spoilage Yeast

Results



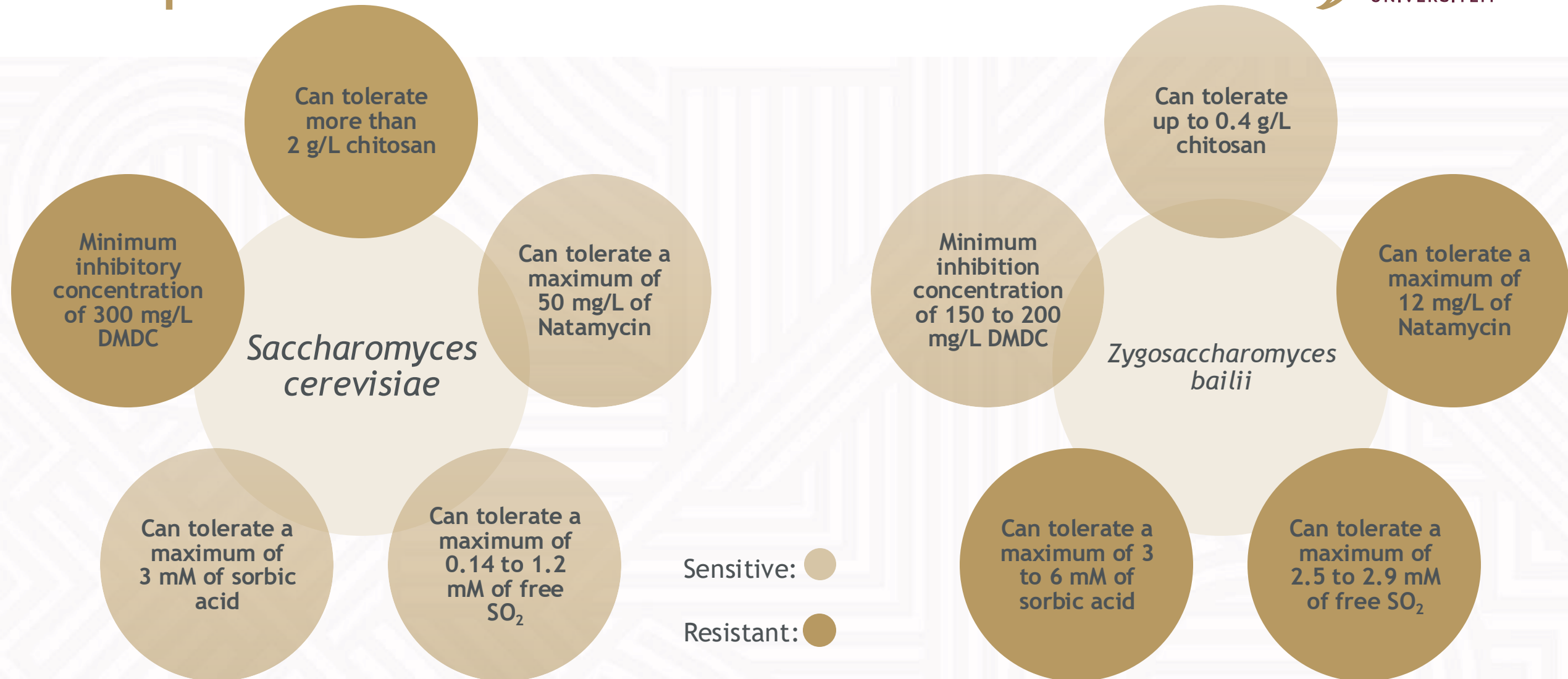
Saccharomyces cerevisiae



Zygosaccharomyces bailii

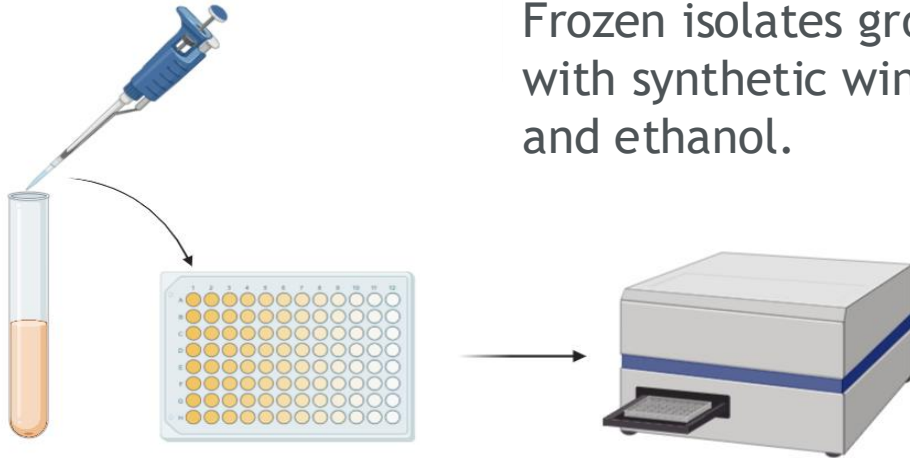
From samples we have analysed, 46% are *S. cerevisiae* and 54% are *Z. bailii*.

Resistance of spoilage organisms to commonly used preservatives



(Meena *et al.*, 2021), (Zalazar *et al.*, 2019), (Bağder Elmacı *et al.*, 2015), (Costa *et al.*, 2008), (Warth, 1985), (Martorell *et al.*, 2007)

Resistance evaluation of isolated yeast



Frozen isolates grown in broth and inoculated into microplate with synthetic wine and varying concentrations of preservatives and ethanol.

Received: 8 July 2021 | Accepted: 6 October 2021 | Published: 21 October 2021
DOI:10.20870/oeno-one.2021.55.4.4809

Yeast stress and death caused by the synergistic effect of ethanol and SO₂ during the second fermentation of sparkling wines

✉ Bruno Cisilotto^{1,2}, Fernando Joel Scariot¹, Luisa Vivian Schwarz¹, Ronaldo Kauê Mattos Rocha¹, Ana Paula Longaray Delamare¹ and Sergio Echeverrigaray¹

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² Federal Institute of Education, Science and Technology of Rio Grande do Sul (IFRS), Campus Bento Gonçalves, Brazil

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✉ Associate editor: Patricia Taillandier

Frequently shown in literature that ethanol works in synergy with many food preservatives.

(Cisilotto *et al.*, 2021)

Ethanol (% v/v)	Total SO ₂ (mg/L)	Sorbic acid (mg/L)	Natamycin (mg/L)	DMDC (mg/L)	Chitosan (g/hL)
0%	100	70	5	70	3
7%	100	70	5	70	3
13%	100	70	5	70	3
0%	200	130	15	130	7
7%	200	130	15	130	7
13%	200	130	15	130	7
0%	250	200	30	200	10
7%	250	200	30	200	10
13%	250	200	30	200	10

Resistance trials:

Ethanol and SO₂

Ethanol and Sorbic acid

Ethanol and DMDC

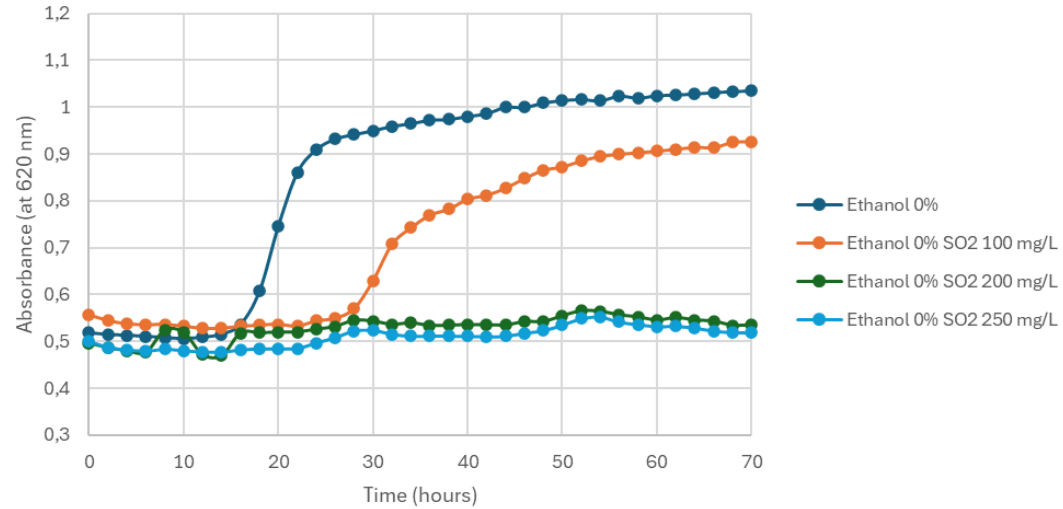
Ethanol and Natamycin

Ethanol and Chitosan

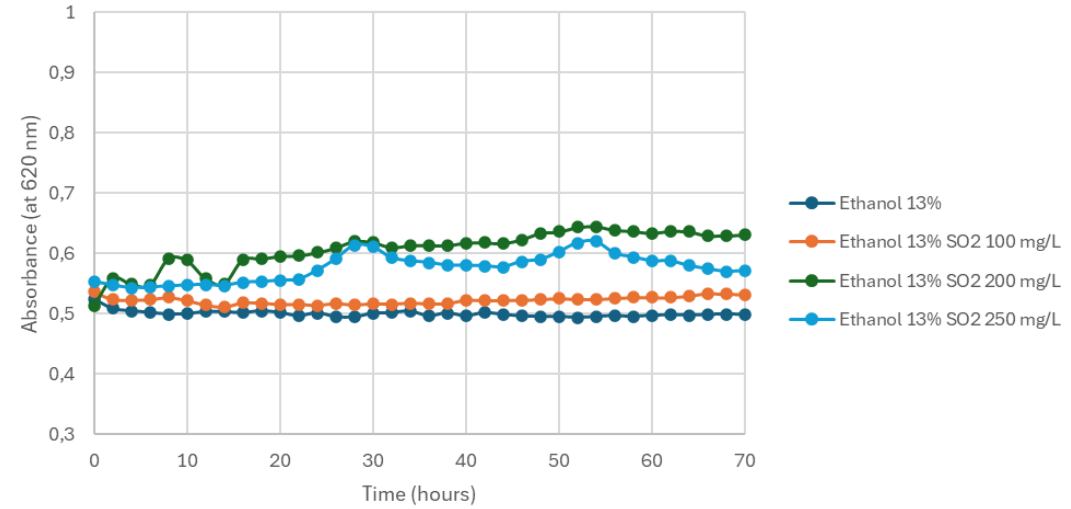
Results

Zygosaccharomyces bailii: Ethanol and SO₂ - The impact of ethanol

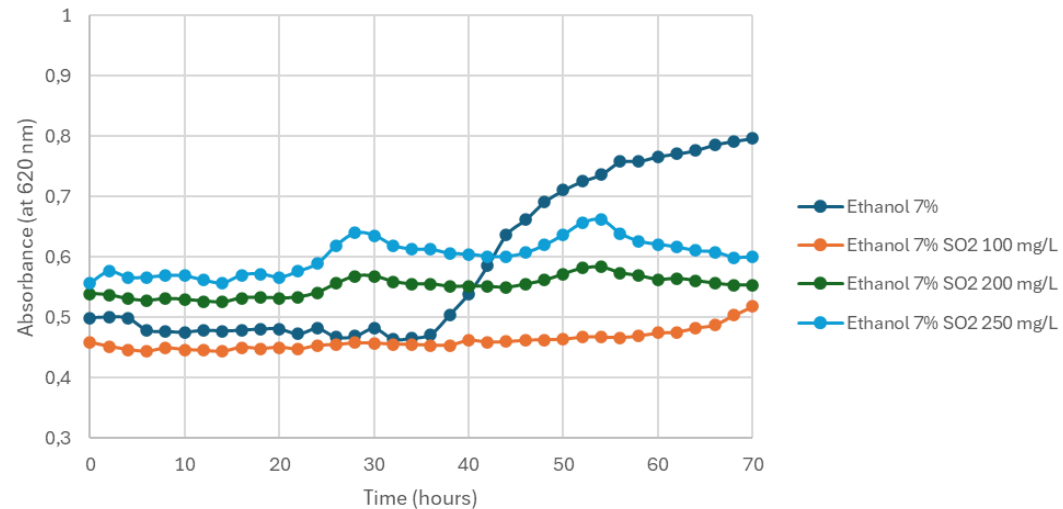
Zygosaccharomyces bailii A
0% Ethanol-SO₂



Zygosaccharomyces bailii A
13% Ethanol-SO₂



Zygosaccharomyces bailii A
7% Ethanol-SO₂

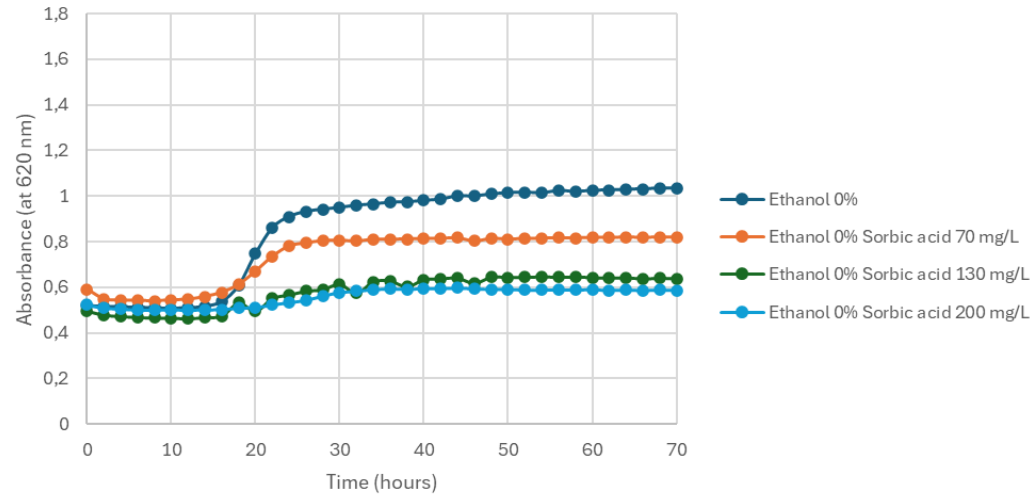


Conclusion:

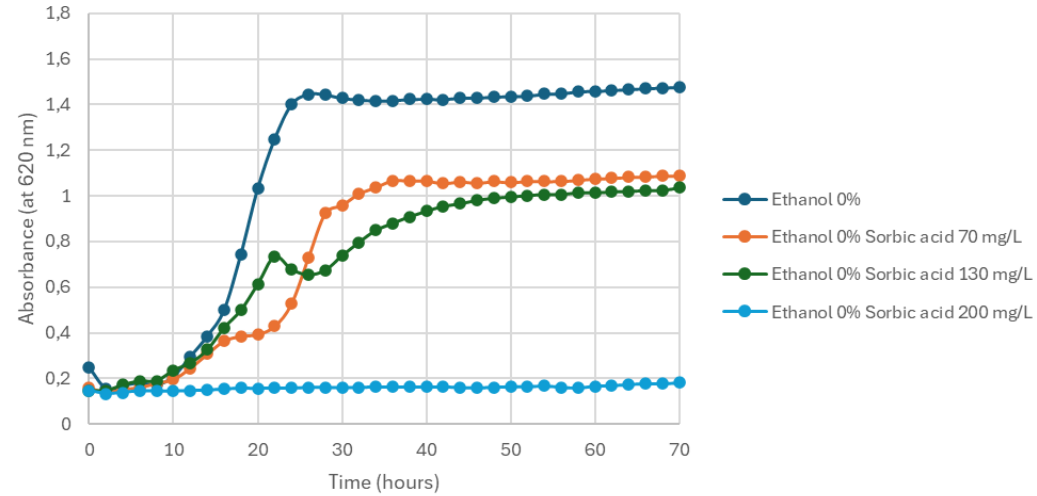
- Increasing the concentration of ethanol, improves the efficacy of SO₂.
- The same trend is seen for many other preservatives tested.

Zygosaccharomyces bailii: Ethanol and Sorbic acid - Strain differences

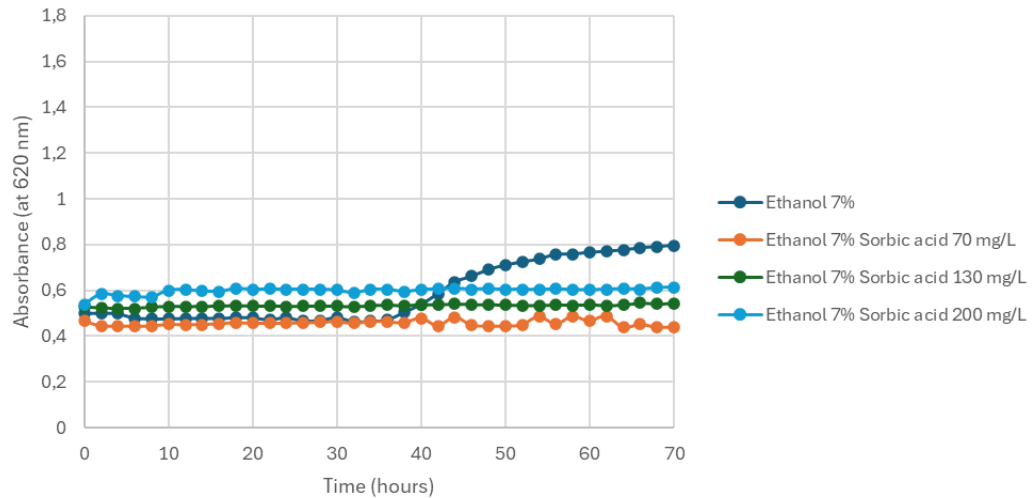
Zygosaccharomyces bailii A
0% Ethanol-Sorbic acid



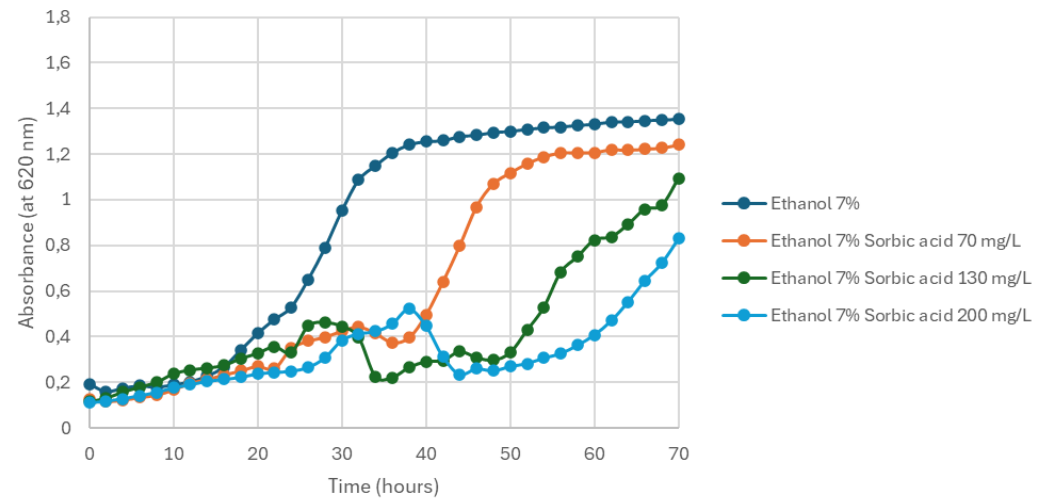
Zygosaccharomyces bailii B
0% Ethanol-Sorbic acid



Zygosaccharomyces bailii A
7% Ethanol-Sorbic acid



Zygosaccharomyces bailii B
7% Ethanol-Sorbic acid

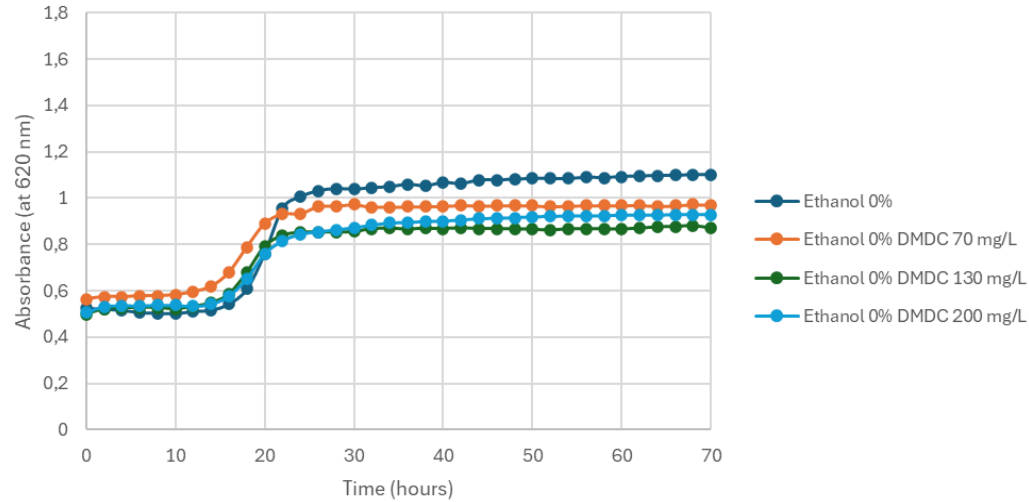


Conclusion:

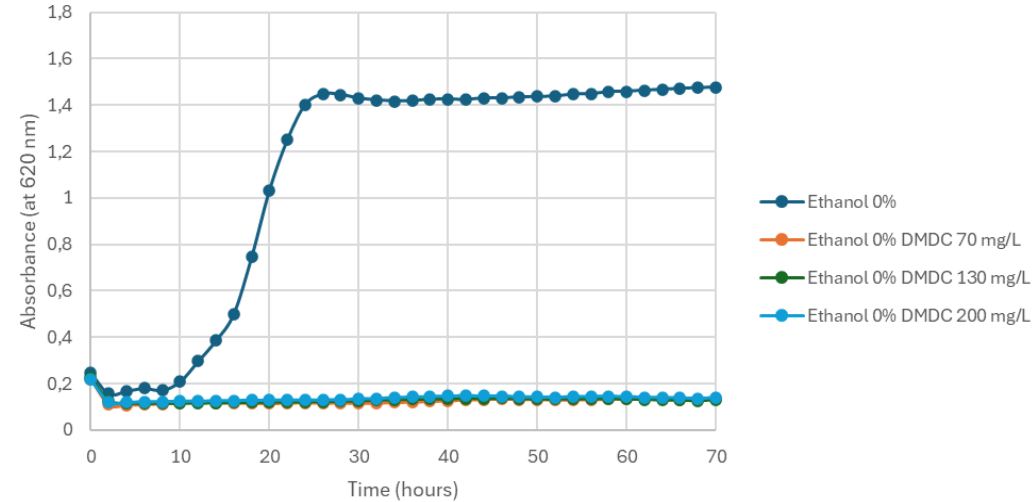
- An important factor contributing to preservative resistance is strain differences.
- Strains of the same species do not necessarily tolerate the same concentrations of preservatives.

Zygosaccharomyces bailii: Ethanol and DMDC - Strain differences

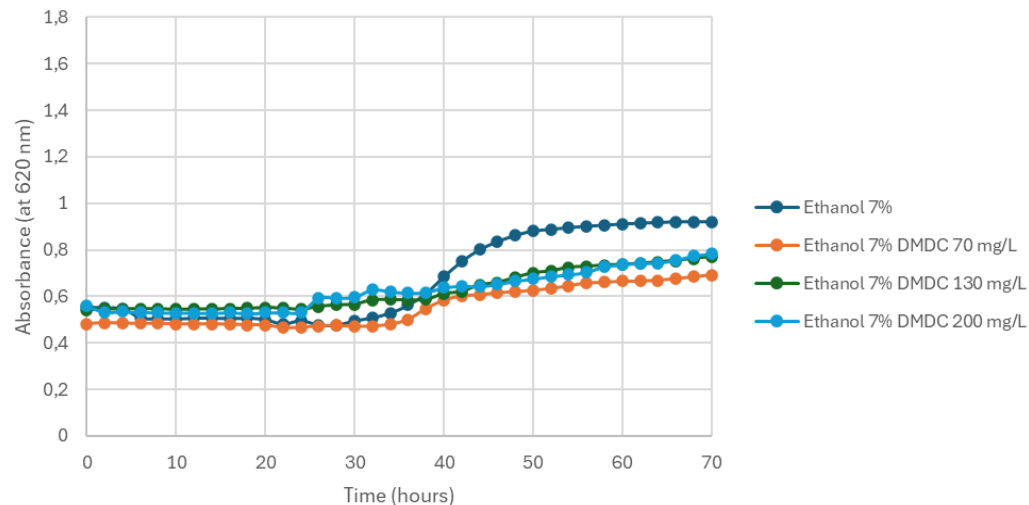
Zygosaccharomyces bailii D
0% Ethanol-DMDC



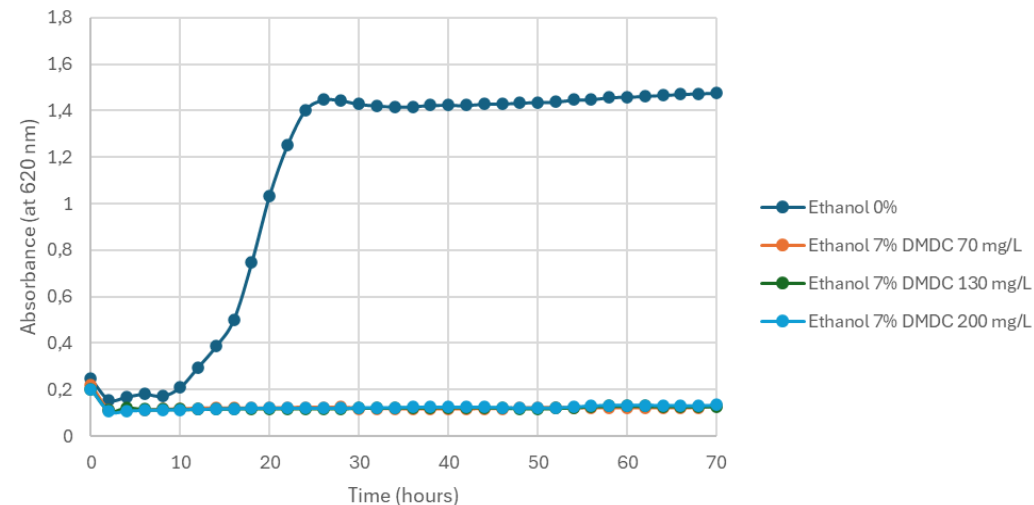
Zygosaccharomyces bailii B
0% Ethanol-DMDC



Zygosaccharomyces bailii D
7% Ethanol-DMDC



Zygosaccharomyces bailii B
7% Ethanol-DMDC

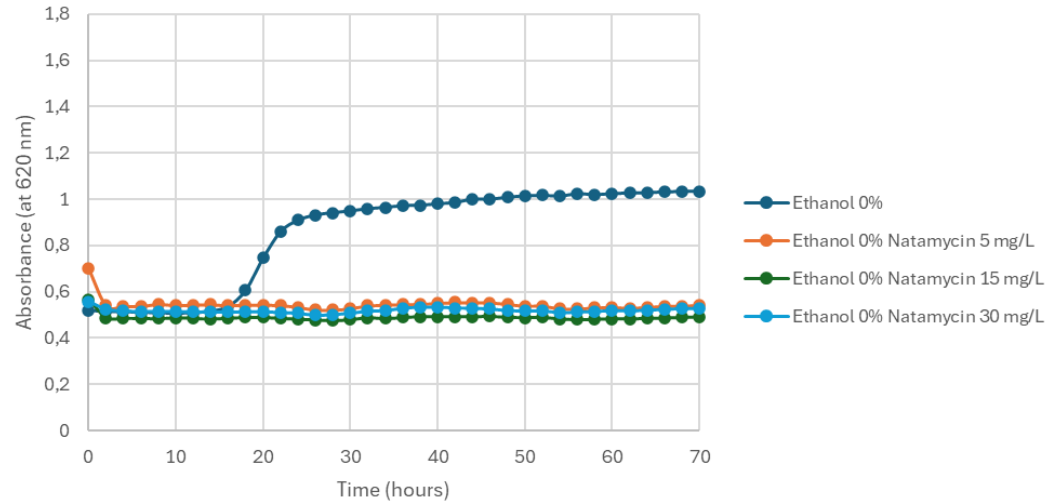


Conclusion:

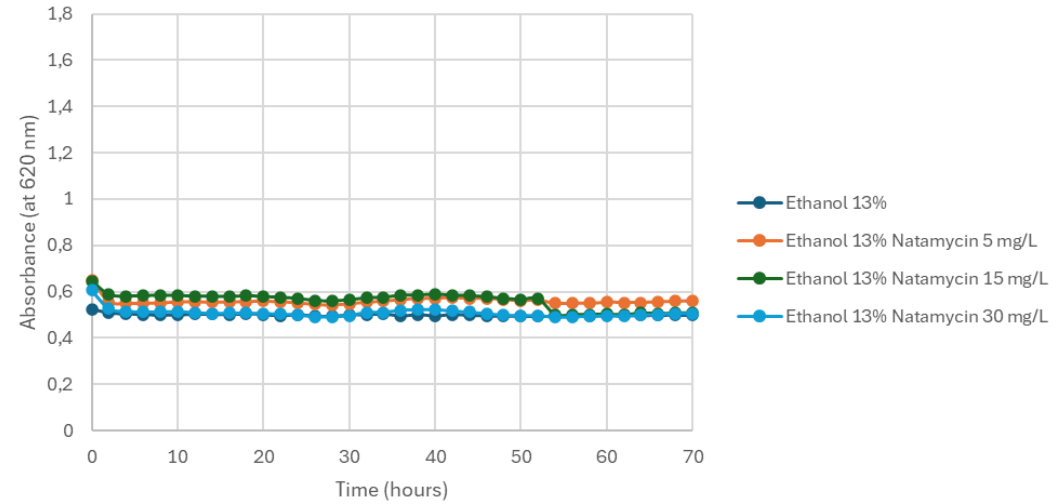
- DMDC shows high efficacy which is aided by ethanol.
- Strain differences in resistance are evident.

Zygosaccharomyces bailii: Ethanol and Natamycin

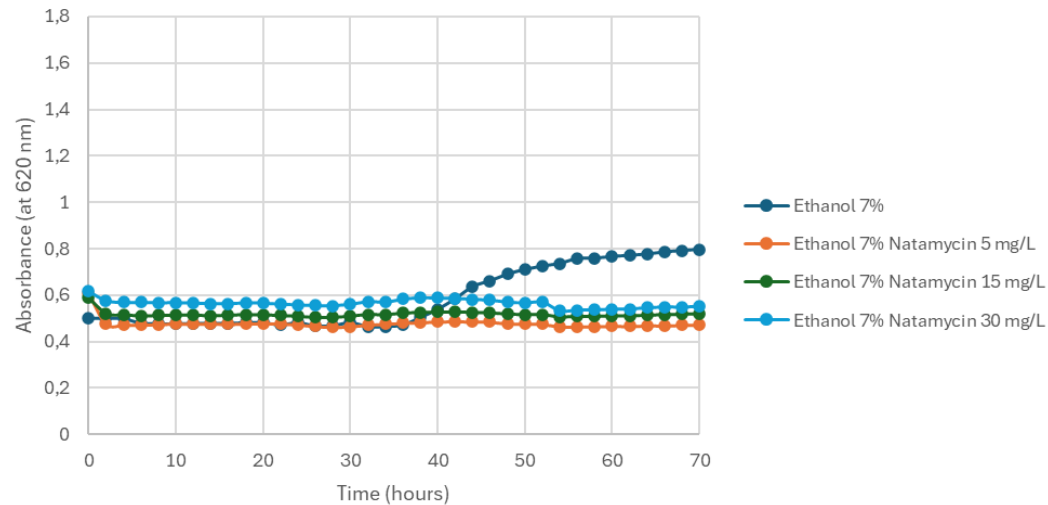
Zygosaccharomyces bailii A
0% Ethanol-Natamycin



Zygosaccharomyces bailii A
13% Ethanol-Natamycin



Zygosaccharomyces bailii A
7% Ethanol-Natamycin

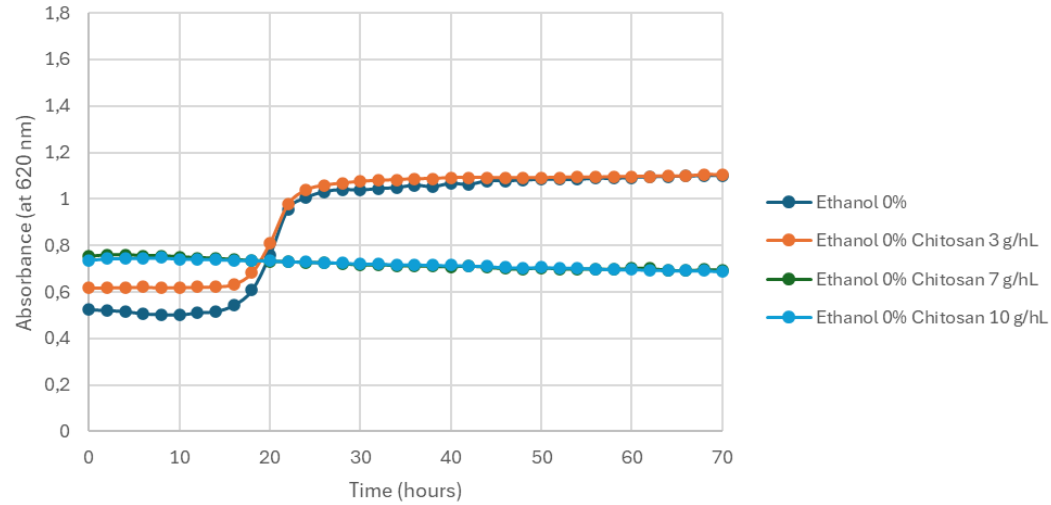


Conclusion:

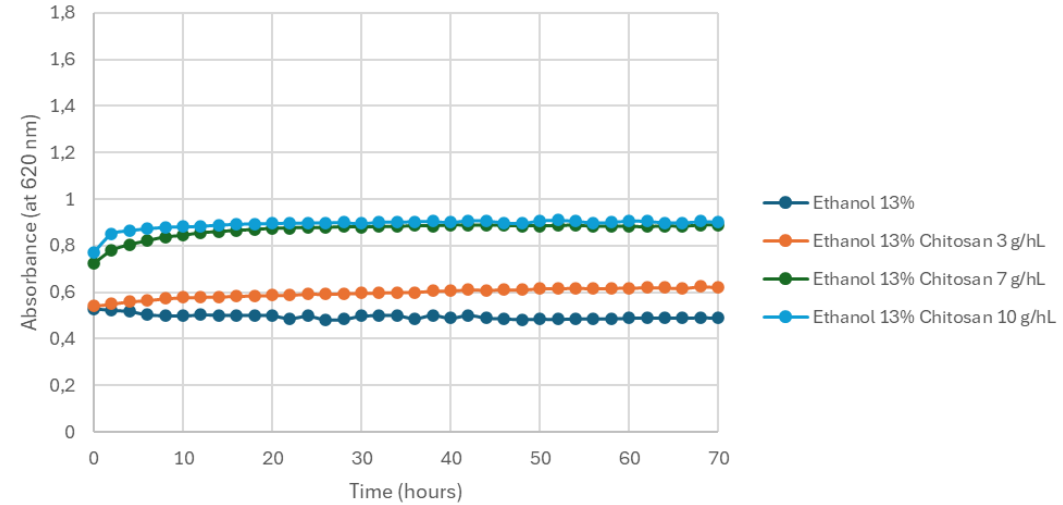
- Natamycin is a highly effective antifungal agent even at low concentrations

Zygosaccharomyces bailii: Ethanol and Chitosan

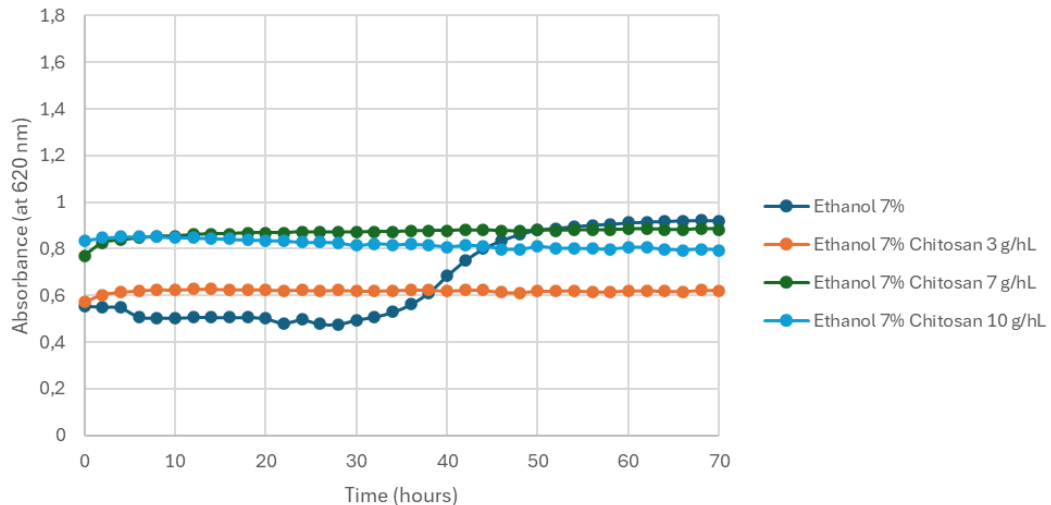
Zygosaccharomyces bailii D
0% Ethanol-Chitosan



Zygosaccharomyces bailii D
13% Ethanol-Chitosan



Zygosaccharomyces bailii D
7% Ethanol-Chitosan



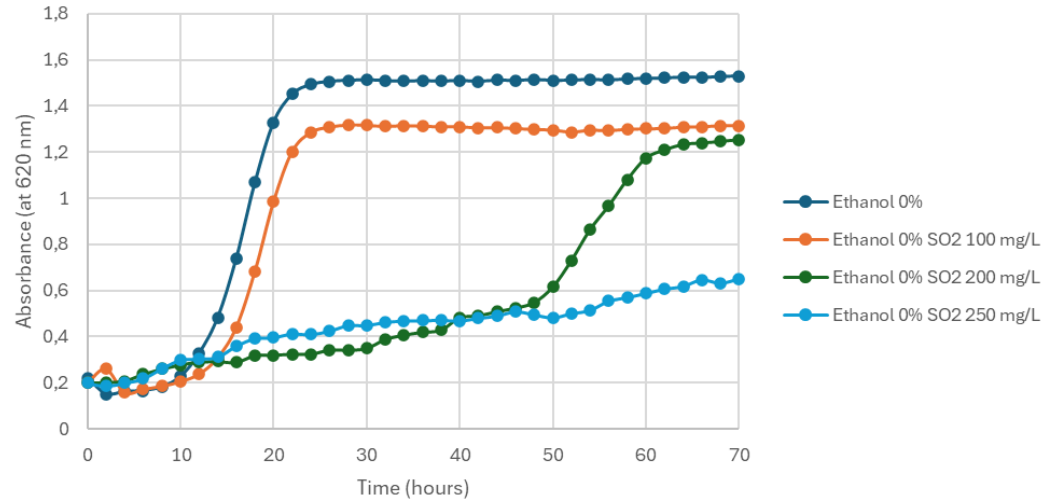
Important to note:
Growth curves with Chitosan present above the control in absorbance. This is because of light scattering/absorbance from chitosan. The key insight lies in the shape of the curve.

Conclusion:

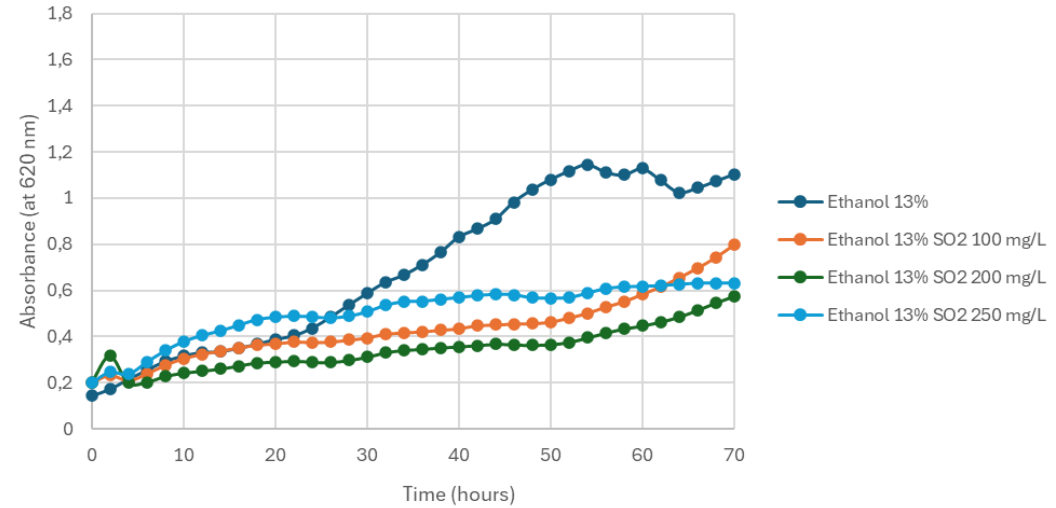
- The antifungal action of Chitosan is improved with ethanol.

Saccharomyces cerevisiae: Ethanol and SO₂ - Species differences

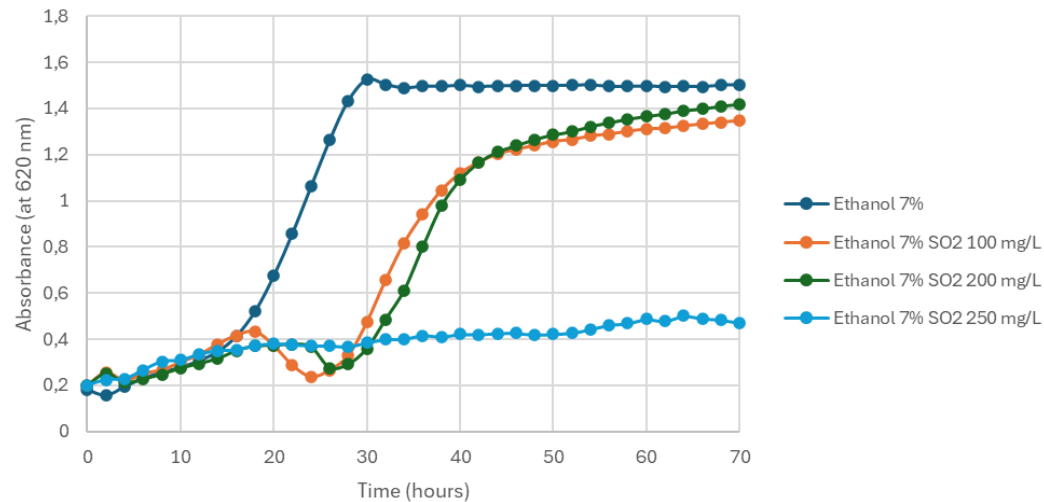
Saccharomyces cerevisiae A
0% Ethanol-SO₂



Saccharomyces cerevisiae A
13% Ethanol-SO₂



Saccharomyces cerevisiae A
7% Ethanol-SO₂



Conclusion:

- Saccharomyces cerevisiae* is well reported as a highly tolerant species of yeast which can withstand high concentrations of ethanol and SO₂ which has been further shown by these results.

Conclusion

The high risk production processes of low- and no-alcohol wine impose a threat to the microbial stability of its products.

Zygosaccharomyces bailii and *Saccharomyces cerevisiae* are dominant spoilage microbes in low- and no-alcohol wine and show high resistance to commonly used preservatives.

According to these results, DMDC, Natamycin and Chitosan show strong inhibition of these microbes but strain differences can be observed.

From these results, one preservation agent may not provide optimal protection in these matrices. Combinations of additives and physical methods are critical to controlling spoilage.

eSurvey: Risk Assessment of Low and No Alcohol Wine

The eSurvey is a critical aspect of my research to determine:

- the impact of spoilage events
- the specific nature of spoilage (source and species)
- optimal strategies to reduce the occurrence of spoilage

Risk Assessment of Low and No Alcohol Wine

NEXT

Acknowledgments



SAGWRI South African
Grape and Wine
Research Institute



SOUTH AFRICA WINE
discover diversity in a glass

Prof. Maret Du Toit

Lynn Engelbrecht

Antonella Fontanel

Stellenbosch University Central
Analytical Facility Sequencing Unit

SA Wine

SAGWRI



SOUTH AFRICA WINE
discover diversity in a glass

SAGWRI
South African Grape and
Wine Research Institute



Stellenbosch
UNIVERSITY
IYUNIVESITHI
UNIVERSITEIT

Thank you
Enkosi
Dankie



QR code for participation