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Beyond Sugar: Yeast Vitamin Requirements in Wine Fermentation

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Photo by Stefan Els

Introduction

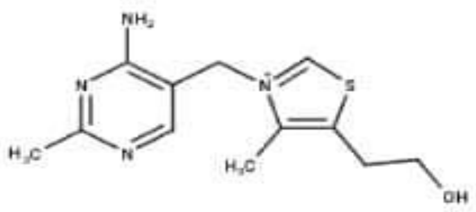
- Yeasts require nutrients during fermentation.
- Nitrogenous compounds
- Carbohydrates
- Lipids
- **Vitamins**
- Various minerals
- The nutrient composition of grape-must significantly influences yeast metabolism, consequently impacting fermentation performance and therefore the quality of wine produced.
- Focus on nitrogen deficiency and glucose/fructose ratio of must.
- Many other factors contribute to fermentation performance.

(Walker, 1998; Julien et al., 2017)

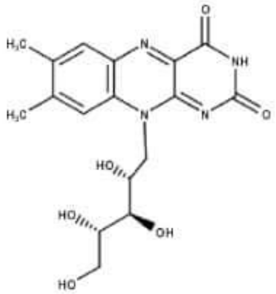
Vitamins

- Vitamins are micronutrients which are essential for cellular physiology.
- Specifically, B-vitamins which are water-soluble vitamins present within grape-musts.
- Despite musts containing these vitamins, deficiency can lead to sluggish fermentations due to decreased:
  - Growth rates
  - Metabolic activity
  - Stress tolerance

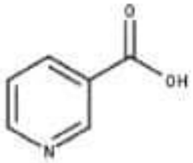
Thiamine (B1)



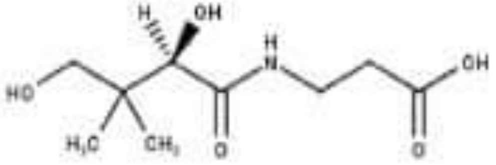
Riboflavin (B2)



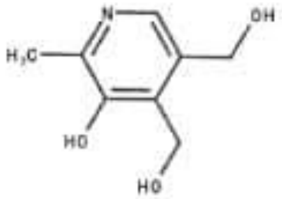
Niacin (B3)



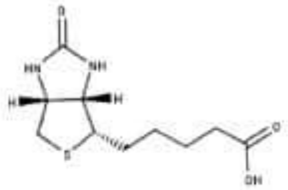
Pantothenic Acid (B5)



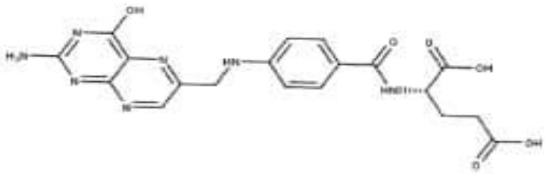
Pyridoxine (B6)



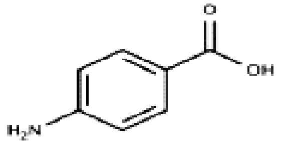
Biotin (B8)



Folic Acid (B9)



*p*ABA (B10)



Why would a deficiency lead to sluggish fermentations?

- B vitamins are converted to metabolically active forms, where they act as co-enzymes by binding to an enzyme and assist in catalysing biochemical reactions.

Vitamins that act as co-enzymes:

- Thiamine (B1) – Coenzyme required for the decarboxylation of pyruvate, enabling yeast to produce key metabolites such as ethanol and acetate.
- Pyridoxine (B6)- Coenzyme in amino acid metabolism.
- Biotin (B8)- Coenzyme involved in carboxylation.

Vitamins that are metabolic precursors for co-enzymes:

- Nicotinic acid (B3)
- Pantothenic acid (B5)
- *p*ABA (B10)

Additional Functions:

- Membrane Integrity.
- Cellular Signaling and protection.
- Respiration.
- Regulate the formation of alcohols and esters.

(Perli, 2020; Evers et al., 2021; Evers et al., 2023)

Biosynthesis vs Uptake

- Yeasts are capable of *de novo* synthesis of various vitamins but may prioritise exogenous uptake for various reasons e.g.: The presence/ absence of O_2
- Some yeast species cannot biosynthesise their required vitamins.

  e.g.: *S. cerevisiae* cannot biosynthesise Biotin(B8).
- Different yeast species have varying essential vitamin requirements.

- Despite various yeast species exhibiting differences in essential vitamin requirements, only thiamine can be supplemented into fermentations, legally.

(Perli, 2020)

The vitamin requirements of wine yeasts

- With mixed and sequential inoculations being used more commonly in wine-making, we may introduce nutrient deficiencies such as the vitamin content of must due to individual requirements, ultimately impacting yeast performance.
- Since, most research has focused on *S. cerevisiae* and the more popular vitamin – Thiamine. Little information is available on non-*Saccharomyces* yeast and other B vitamins.

➢ Most recent research: Evers et al., 2023- Where vitaminic preferences are observed among wine species, with differences in vitamin assimilation compared to *S. cerevisiae*.

- This highlights the importance of investigating the nutrient requirements of non-*Saccharomyces* yeasts commonly used in fermentations.

(Labuschagne, 2021, Evers, 2021)

Aims & Objectives

Despite vitamins playing a crucial role in yeast metabolism:

- Little information is available on the vitamin requirements of yeast strains, especially non-*Saccharomyces* species.
- **Aim:** This research project assessed the vitamin requirements of wine yeasts, as well as assessed how these vitamins affected metabolic profiles.
- **Objective: Assessed the ability of wine yeasts to grow in the presence/absence of B-vitamins to determine possible auxotrophies. Determined the impact of varying vitamin concentrations on fermentation performance and metabolic footprint.**

Yeast vitamin requirement: Species & Vitamin Selection

Species: A combination of commercialized strains and strains isolated from grape juice

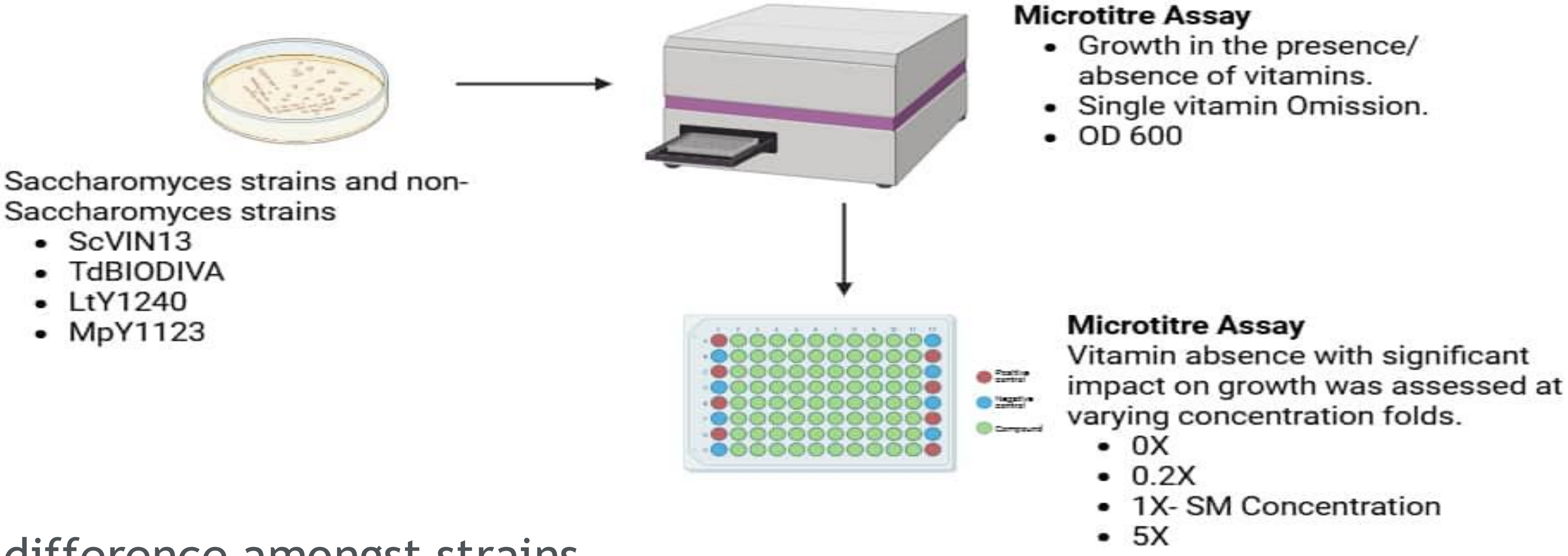
Species	Strain
Saccharomyces cerevisiae (Sc)	Lalvin® EC1118
	VIN13
	Y1022
Torulaspora delbrueckii (Td)	Biodiva™ TD291
	LO544
	Y930
Lachancea thermotolerans (Lt)	Laktia™
	IWBT Y1240
	Y513
Metschnikowia pulcherrima (Mp)	Flavia™ Y956
	IWBT Y1123

Vitamins of interest: Vitamins within Synthetic Must (Bely, 1990;Henschke and Jiranek, 1993)

Vitamins in Synthetic Grape Must	Vitamin Concentration (1X)	0.2X	5X
Myo-inositol (mg/L)	100	20	500
Biotin (mg/L)	0.13	0.03	0.65
Thiamine hydrochloride (mg/L)	0.5	0.10	2.5
Nicotinic acid (mg/L)	2	0.4	10
Calcium pantothenate (mg/L)	1	0.2	5
Pyridoxine hydrochloride (mg/L)	2	0.4	10

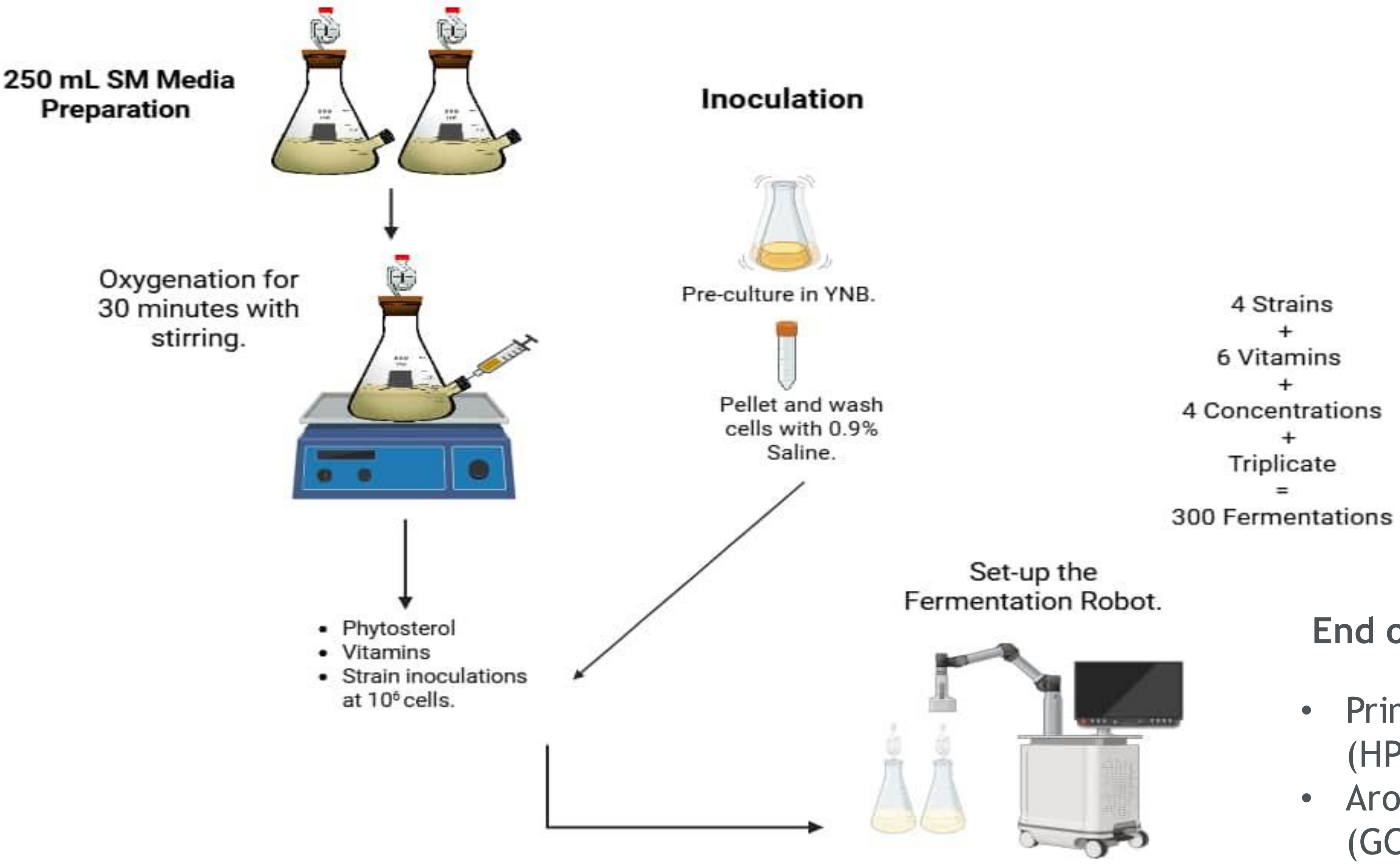
0X- Absence of vitamin

Screening Phase: Assess the ability of yeast strains to grow in the presence/absence of B vitamins



- No difference amongst strains.
- However, there were differences amongst species required vitamins.

Screening Phase: Small-scale fermentations



0X, 1X, 0,2X and 5X

End of Fermentation

- Primary metabolites (HPLC)
- Aroma compounds (GC/MS)

Fermentation Kinetics: *S. cerevisiae*

Table 1: Fermentation rate (rmax) of S. cerevisiae

Species_vitamin	Vitamin_Concentration	Rmax (mean ± SD)
Sc_B1	0X	0.290 ± 0.020
Sc_B3	0X	0.550 ± 0.040
Sc_B5	0X	0.550 ± 0.020
Sc_B6	0X	0.270 ± 0.010
Sc_B8	0X	0.380 ± 0.040
Sc_M	0X	1.450 ± 0.060
Sc_B1	0,2X	2.690 ± 0.100
Sc_B3	0,2X	2.570 ± 0.140
Sc_B5	0,2X	3.010 ± 0.030
Sc_B6	0,2X	3.030 ± 0.080
Sc_B8	0,2X	0.490 ± 0.030
Sc_M	0,2X	2.950 ± 0.130
Sc_All Vitamins	1X	2.660 ± 0.070
Sc_B1	5X	3.010 ± 0.070
Sc_B3	5X	3.020 ± 0.070
Sc_B5	5X	3.080 ± 0.080
Sc_B6	5X	3.040 ± 0.050
Sc_B8	5X	2.940 ± 0.050
Sc_M	5X	2.990 ± 0.020

rmax- the maximum rate of CO_2 production during fermentation.

- 0X Vitamin concentration decreases fermentation rate.
- 5X (Extreme increase) caused an increase in fermentation rate.
- 0,2X has a similar effect to that of the control (1X).

Fermentation Kinetics: *L. thermotolerans*

Table 2: Fermentation rate (rmax) of *L. thermotolerans*

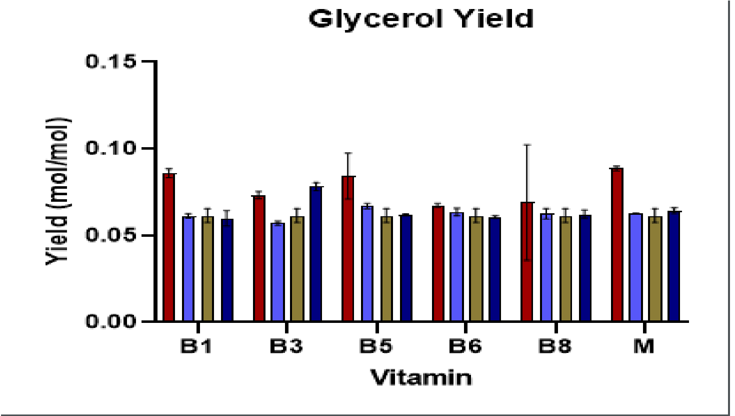
Species_vitamin	Vitamin Concentration	Rmax (mean ± SD)
Lt_B1	0X	0.487 ± 0.028
Lt_B3	0X	0.456 ± 0.021
Lt_B5	0X	0.461 ± 0.014
Lt_B6	0X	0.417 ± 0.022
Lt_B8	0X	0.447 ± 0.004
Lt_M	0X	0.027 ± 0.004
Lt_B1	0,2X	1.776 ± 0.205
Lt_B3	0,2X	1.273 ± 0.020
Lt_B5	0,2X	2.008 ± 0.050
Lt_B6	0,2X	2.076 ± 0.035
Lt_B8	0,2X	0.022 ± 0.007
Lt_M	0,2X	2.054 ± 0.020
Lt_All Vitamins	1X	1.942 ± 0.030
Lt_B1	5X	1.776 ± 0.205
Lt_B3	5X	0.521 ± 0.023
Lt_B5	5X	0.512 ± 0.019
Lt_B6	5X	0.527 ± 0.021
Lt_B8	5X	0.542 ± 0.091
Lt_M	5X	0.503 ± 0.016

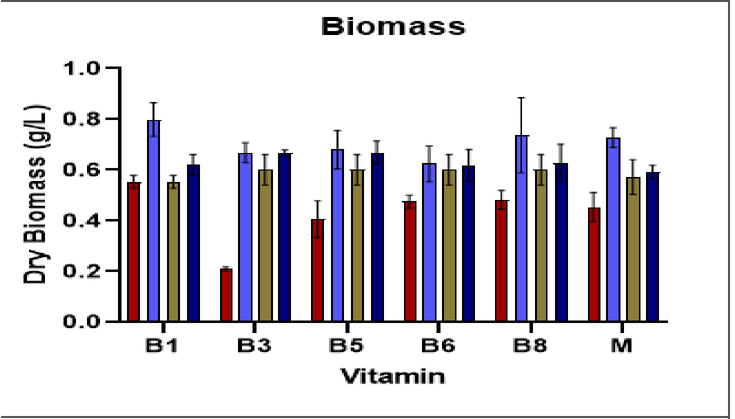
- 0X Vitamin concentration decreases fermentation rate.
- 5X (Extreme increase) caused a decrease in fermentation rate.
- 0,2X has a similar effect to that of the control (1X).

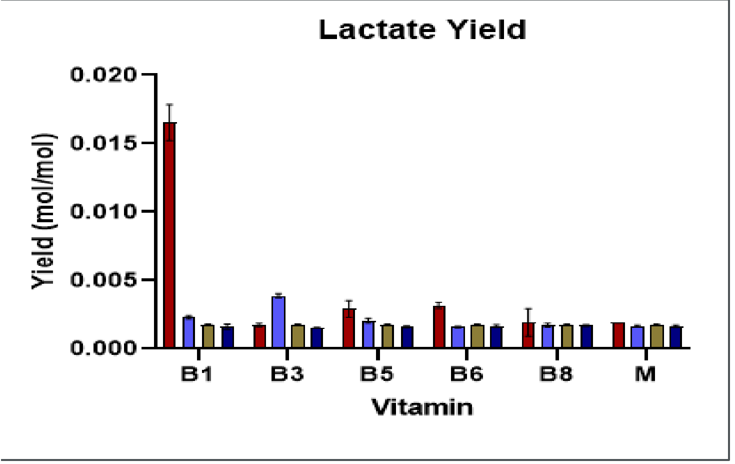
Summary of fermentation kinetics across all species:

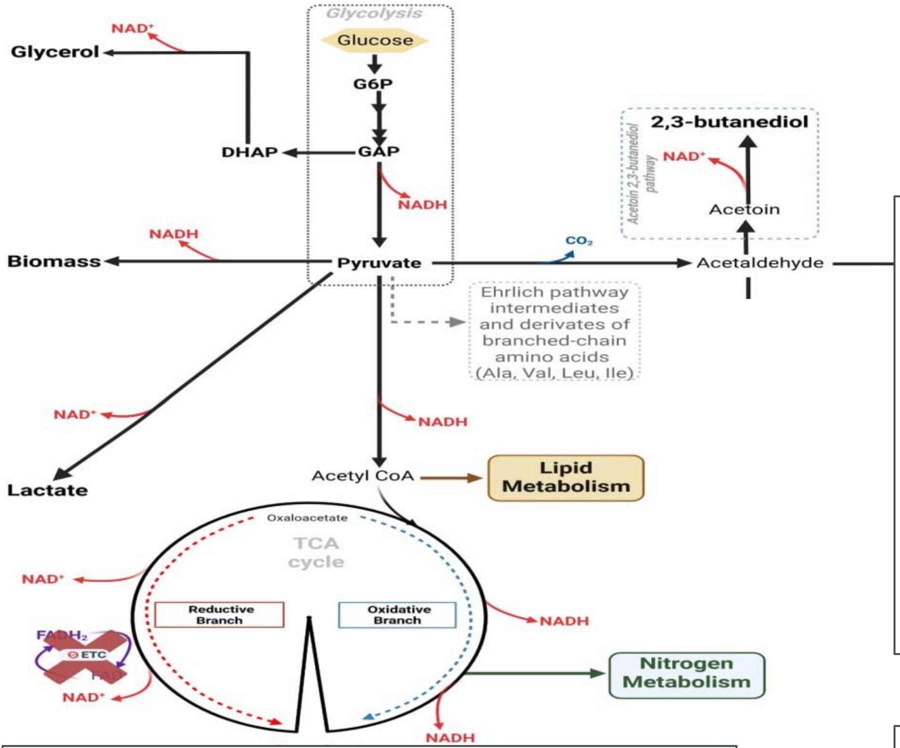
Species	0X	0,2X	5X	Largest Impact	Observations
S. cerevisiae	All vitamins required, except myo-inositol.	Fermentation performance remained the same.	Fermentation performance improved.	B3 & B8	At 5X vitamin concentration fermentation rate increased
T. delbrueckii	All vitamins required.	Fermentation performance remained the same.	Fermentation performance decreased.	B1, B3 & B8	At 5X vitamin concentration fermentation rate decreased
L. thermotolerans	All vitamins required.	Fermentation performance remained the same.	Fermentation performance decreased.	B3, B5 & B6.	At 5X vitamin concentration fermentation rate and performance decreased significantly.
M. pulcherrima	Minor effect.	Fermentation performance increased.	Fermentation performance decreased.	NA	Each vitamin concentration caused a different response.

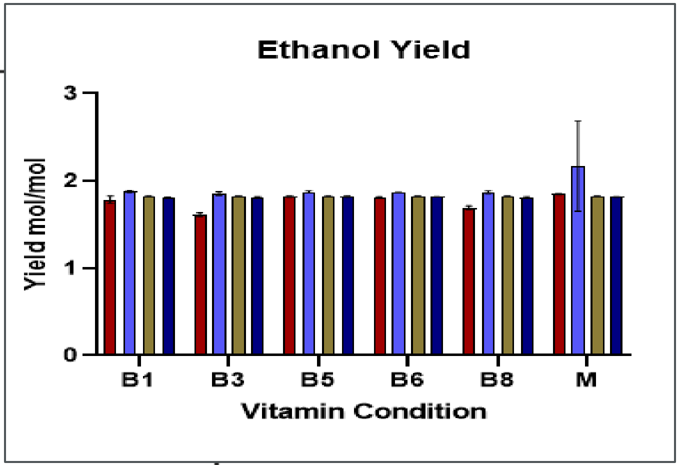
The impact of B vitamins on metabolite production- *S. cerevisiae*

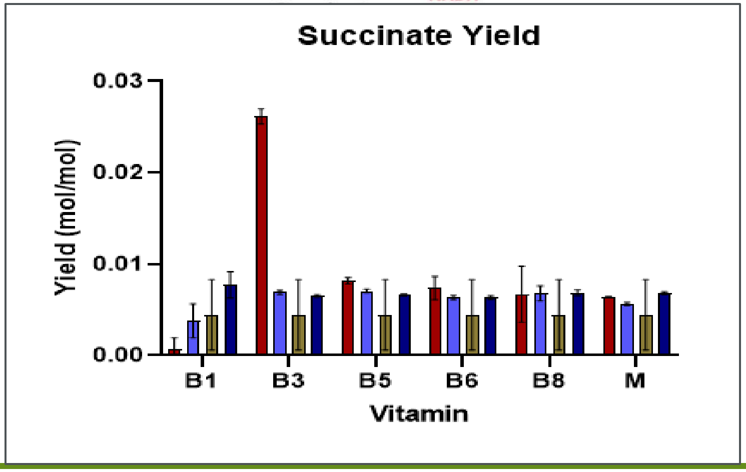


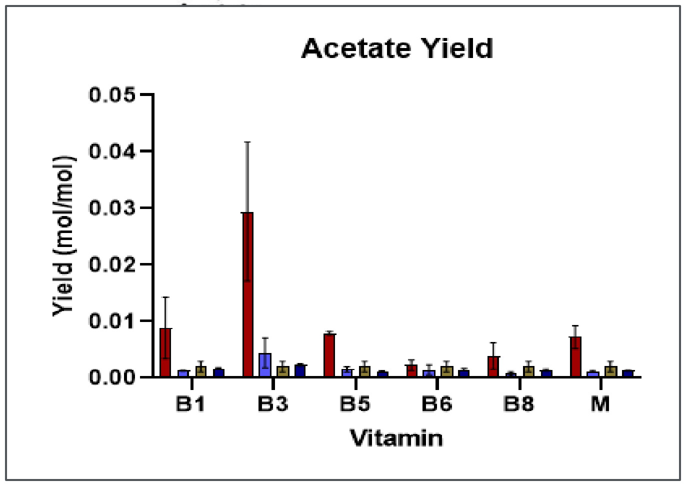






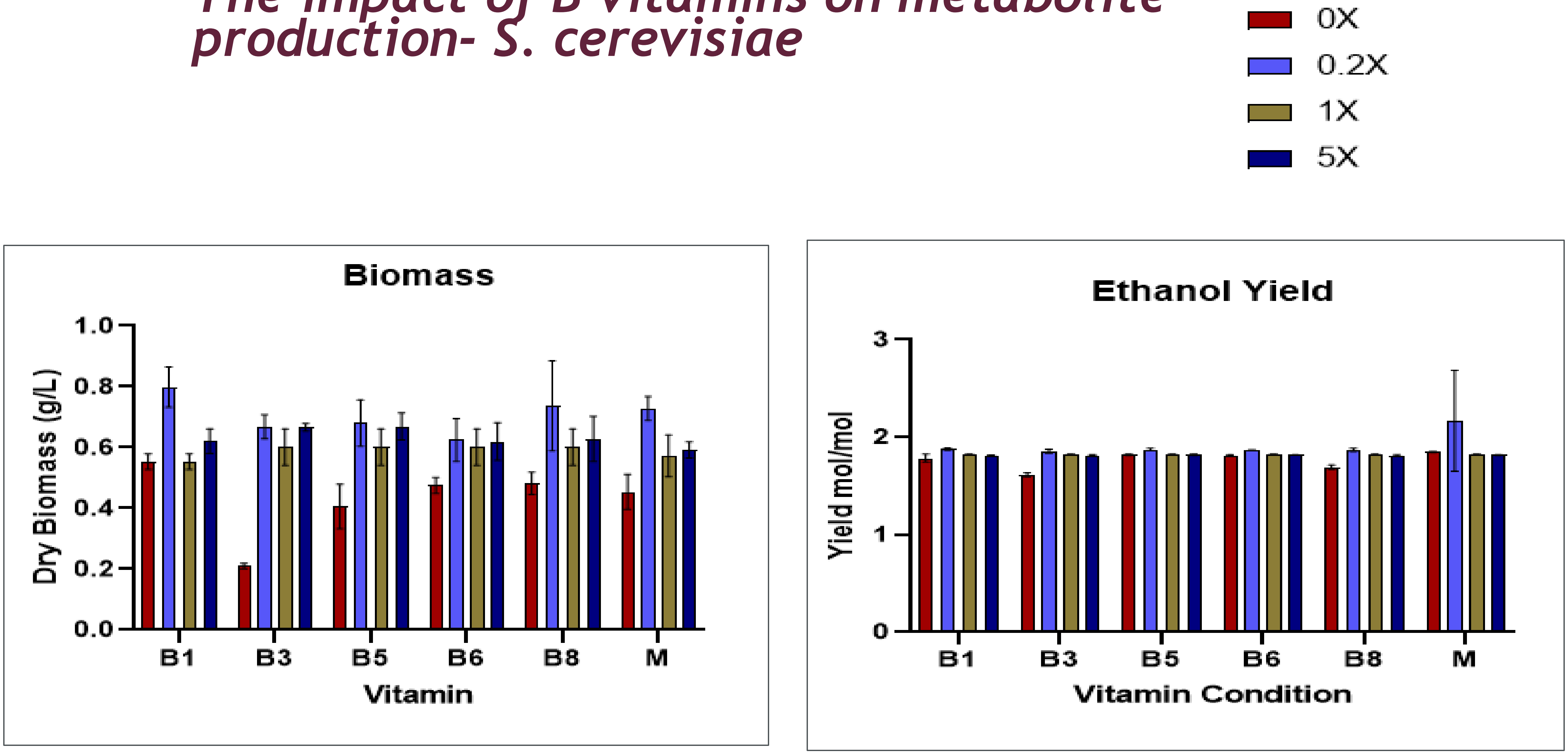




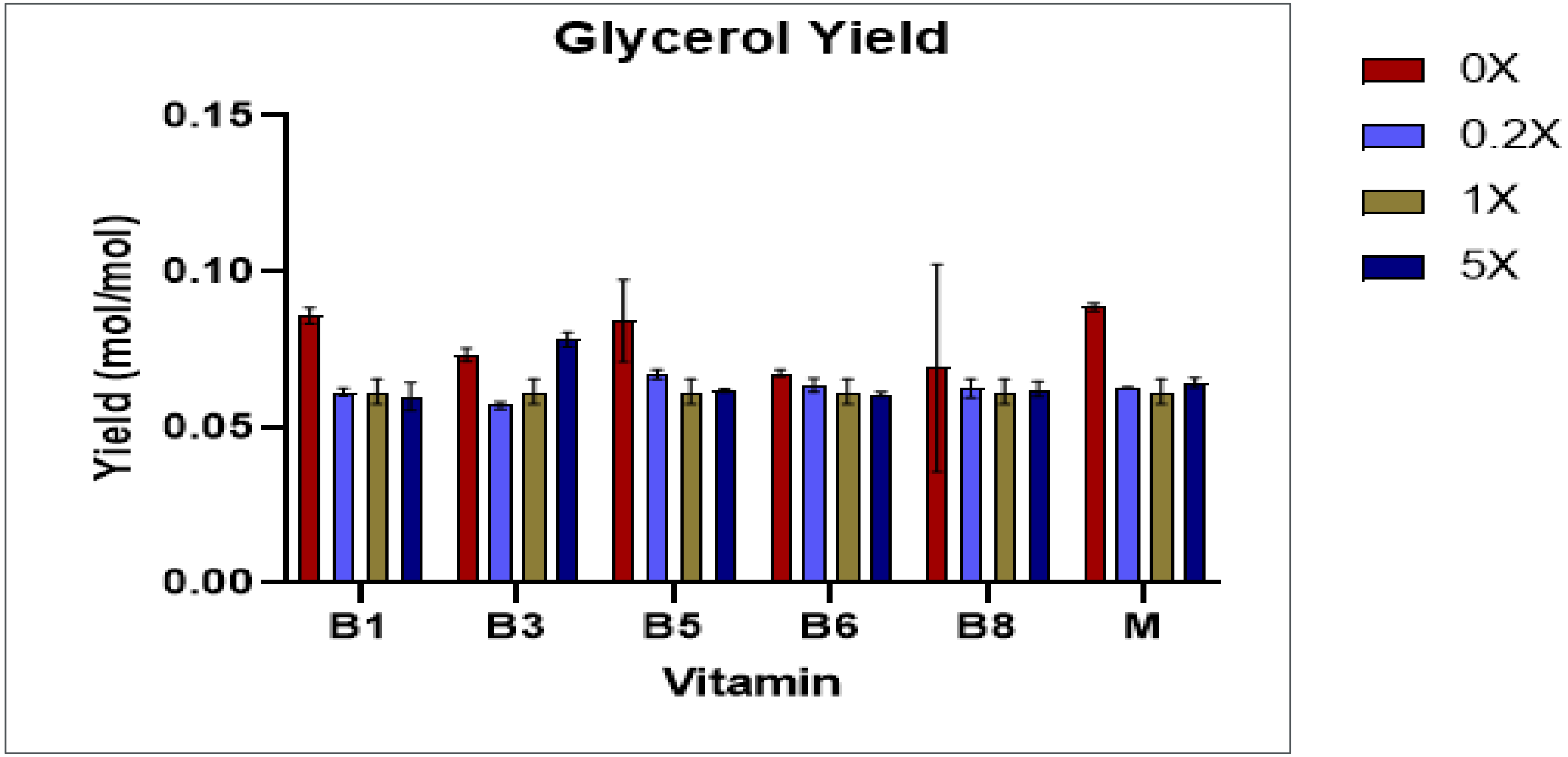


Tyibilika, 2024

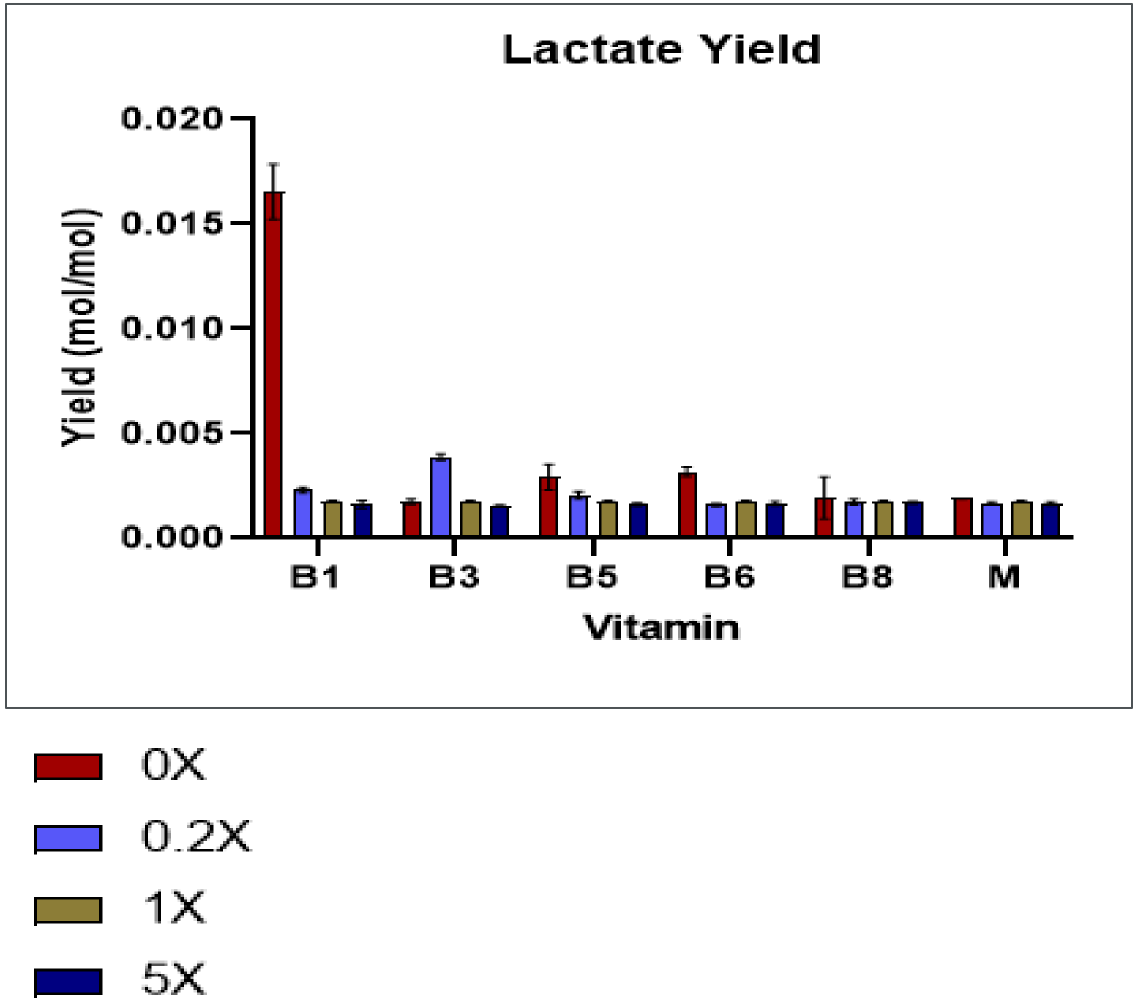
The impact of B vitamins on metabolite production- S. cerevisiae

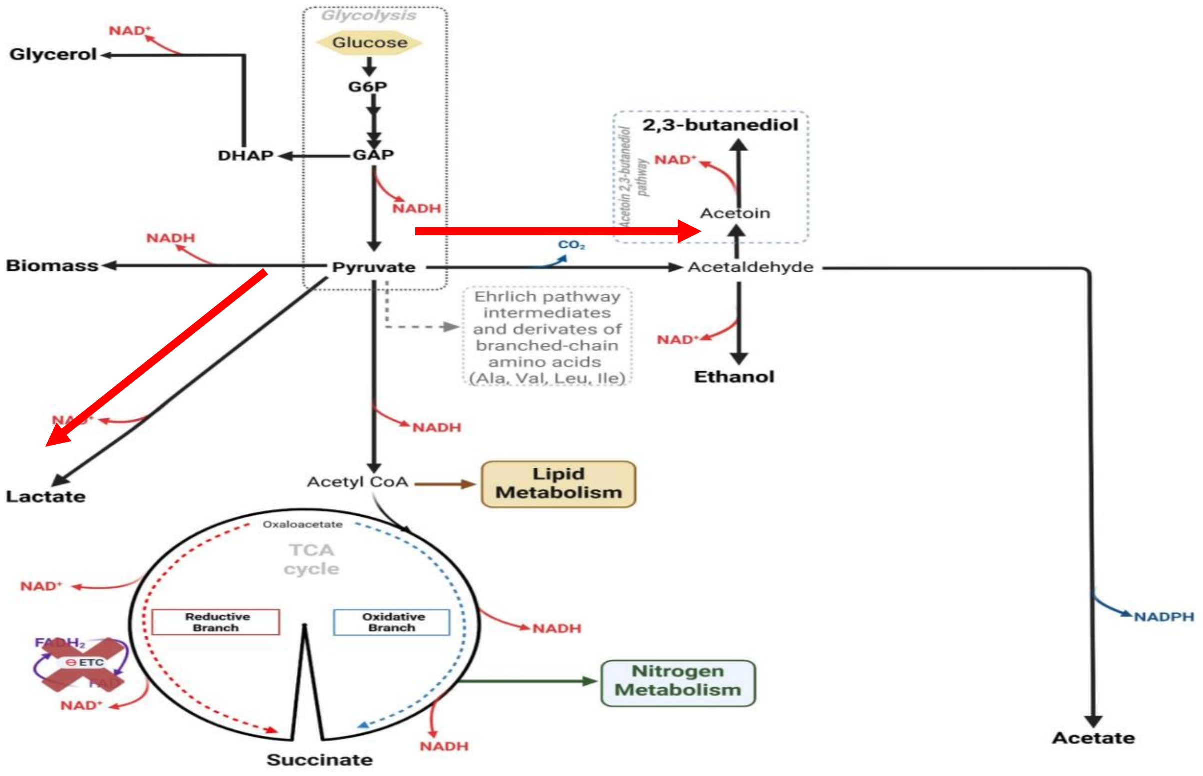


The impact of B vitamins on metabolite production- S. cerevisiae

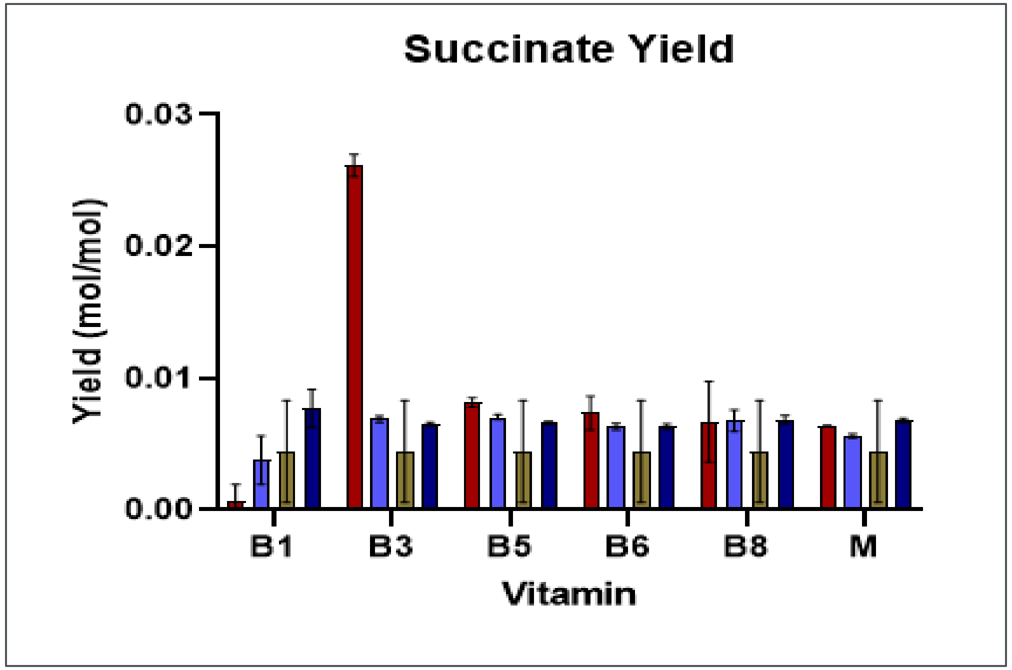


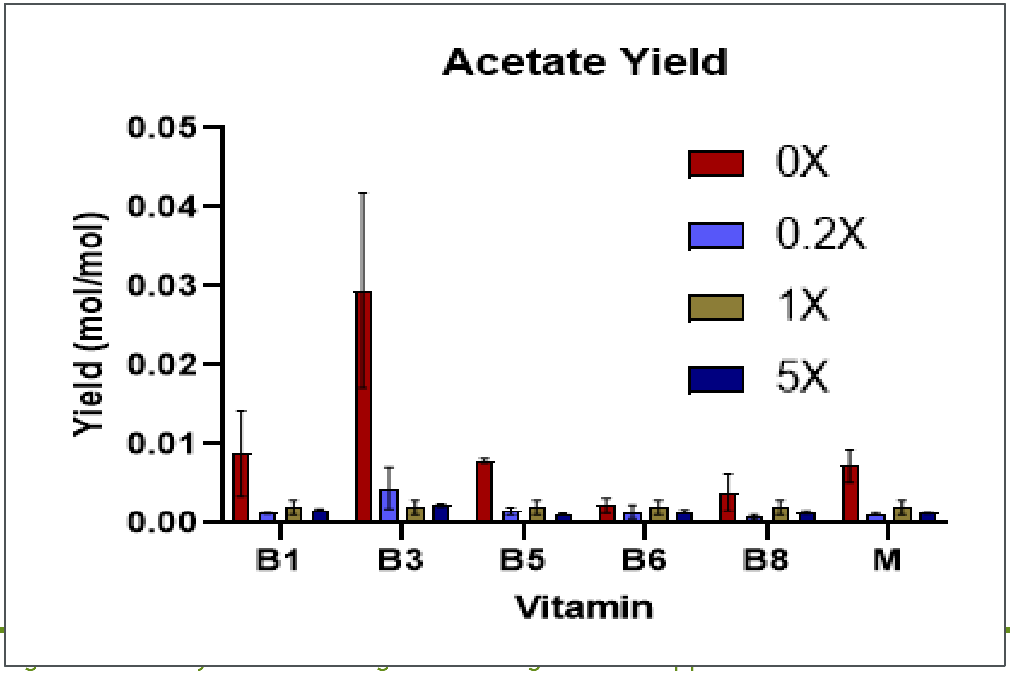
The impact of B vitamins on metabolite production- S. cerevisiae

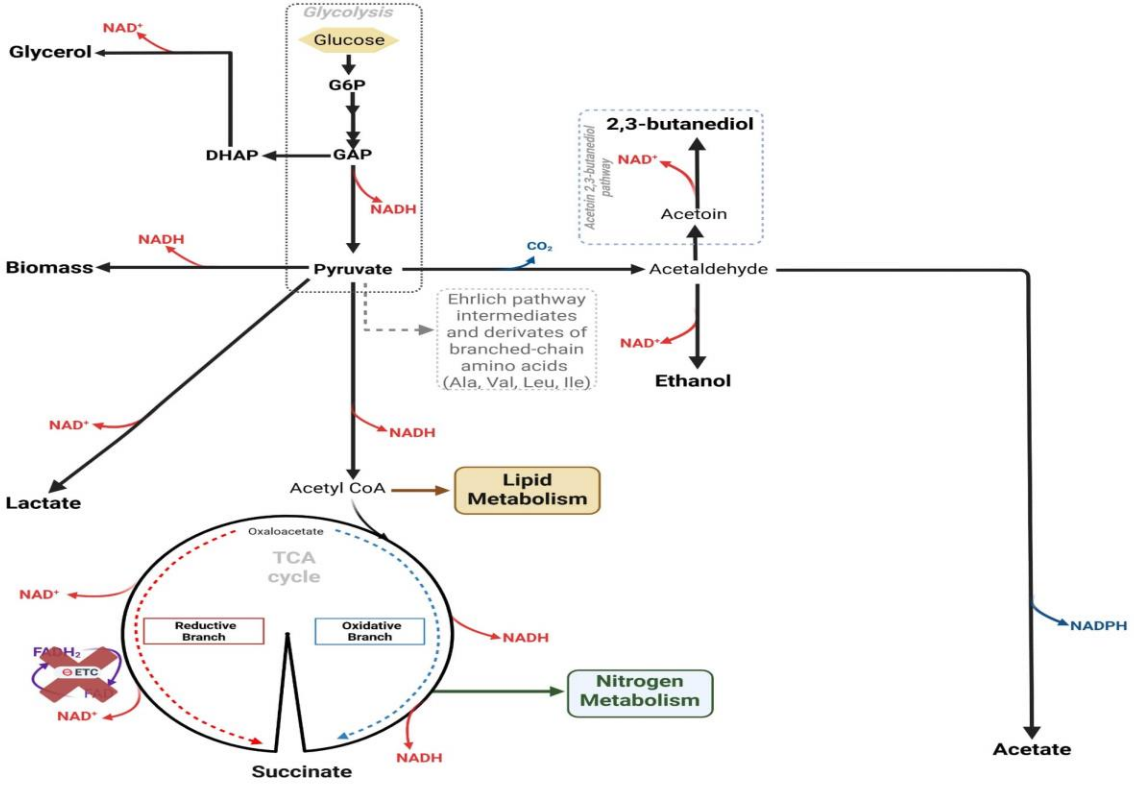




The impact of B vitamins on metabolite production- S. cerevisiae







Summary of metabolic response across all species:

Species	Biomass	Ethanol Yield	Glycerol Yield	Succinate Yield	Acetate Yield	Lactate Yield
S. cerevisiae	↓ at 0X	Unaffected (Except B3 & B8)	↑ at 0X	↑ at 0X B3	↑ at 0X B5 & B8	↑ at 0X B1
T. delbrueckii	↓ at 0X	Unaffected (Except B3 & B8)	↑ at 0X	↑ at 0X B3	↑ at 0X B3	↑ at 0X B1
L. thermotolerans	↓ at 0X & 5X	Unaffected- slight ↓ at 0X & 5X	↑ at 0X & 5X	↑ at 0X B3 & B6	↑ at 0X B5 & B6	NA
M. pulcherrima	↓ at 0X & 5X	Unaffected (minimal)	↑ at 0X B3	↑ at 0X B3	Unaffected	↑ at 0X B1

Conserved trends across all species:

- 0X almost always ↓ kinetics & biomass.
- B3 limitation repeatedly produces ↓ethanol & ↑glycerol, ↑acetate, ↑succinate
- B1-0X → lactate spikes

Vitamins with largest impacts:

- B1
- B3
- Honourable mention: B8- Impacts fermentation kinetics, primary metabolite yields for Sc, Td and Lt.

Species specific metabolic response:

- *L. thermotolerans* and *M. pulcherrima* show sensitivity towards both an absence and extreme increase of vitamin concentration.

- Acetate yield increase differs with vitamins across species
- *S. cerevisiae*: B5 & B8 at 0X
- *T. delbrueckii* : B3 at 0X
- *L. thermotolerans*: B5 & B6 at 0X and 5X
- *M. pulcherrima*: Unaffected

- Note: *L. thermotolerans* strain shows the most divergence in response to vitamin absence and varying concentrations.

Conclusion

- B vitamin availability has species-specific impacts.
- Vitamin limitation can affect fermentation efficiency.
- Non-*Saccharomyces*, specifically *L. thermotolerans* might have a specific/ optimal concentration requirement.
- As a similar trend was observed with *K. marxianus* in a study done by Labuschagne, 2021, where *S. cerevisiae* and *K. marxianus* assimilated thiamine differently.
- Fermentation performance and aroma production improved at a specific concentration of B1 and high concentrations had a high-saturation effect.

Take home message:

- Although important, B1 is not the only required vitamin.
- Importance of vitamins B3, B5 and B8 across all species.
- Additionally to vitamins playing an important role in yeast metabolic activity and species exhibiting vitamin-specific requirements:

- non-*Saccharomyces* yeasts show sensitivity to vitamin availability

This highlights the importance of refining vitamin supplementation strategies in mixed and sequential fermentations to fully harness the oenological potential of non-*Saccharomyces* species. This way we can potentially enhance desirable non-*Saccharomyces* attributes.

Acknowledgements

- SAGWRI researchers.
- UMR SPO, INRAE, Institut Agro, Université de Montpellier, Montpellier, France
- MicroWise
- The Embassy of France in SA & Campus France
- NRF.

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Thank you
Enkosi
Dankie
Merci

Photo by Stefan Els