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Nicotinic acid deficiency impacts wine fermentation efficiency in *Saccharomyces cerevisiae*

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Nicotinic acid (NA), a form of vitamin B3, is a precursor to essential redox cofactors NAD^+ and NADP, which are critical for various metabolic processes. In *Saccharomyces cerevisiae*, the production of these cofactors under anaerobic conditions occurs exclusively by salvaging various forms of vitamin B3, underscoring the significance of NA availability in grape juice. In this study, we investigated the impact of NA deficiency on the fermentation performance of *S. cerevisiae* and the subsequent impact on the metabolic footprint of wine.

Nicotinic acid deficiency negatively impacts alcoholic fermentation kinetics

The fermentation kinetics of *Saccharomyces cerevisiae* EC1118™ (Lallemand Inc, QC, Canada) (Figure 1) were compared in a synthetic grape juice medium containing 230 g/L of sugars (half glucose, half fructose), with three different nicotinic acid (NA) concentrations at 25°C. This range encompassed deficiency (0.1 mg/L), average (2.0 mg/L) and excess (4.0 mg/L) levels of the vitamin typically measured in grape must, although actual concentrations can vary greatly[1]. While the fermentation kinetics monitored as cumulative weight loss appeared similar in the first 24 h (*i.e.* lag phase and onset of exponential phase), deficient NA levels resulted in slower fermentation and 37.5 % reduction in yeast biomass production. Interestingly, excess levels of NA had little impact on the fermentation rate and biomass compared to normal concentrations. Although fermentation was completed with *S. cerevisiae* in this work, another recent study showed that NA limitation in non-*Saccharomyces* species such as *Kluyveromyces marxianus* and *Lachancea thermotolerans* further reduced fermentation rates and altered metabolite production[2]. On the other hand, in *Starmerella bacillaris*, NA limitation had minimal impact on early fermentation rates but resulted in reduced metabolic activity during the stationary phase, leading to premature stuck fermentation[2]. Although these results were mostly obtained in specific strains, these findings suggest species-specific differences in response to this nutrient limitation as well the resulting unique metabolic footprints in each yeasts.

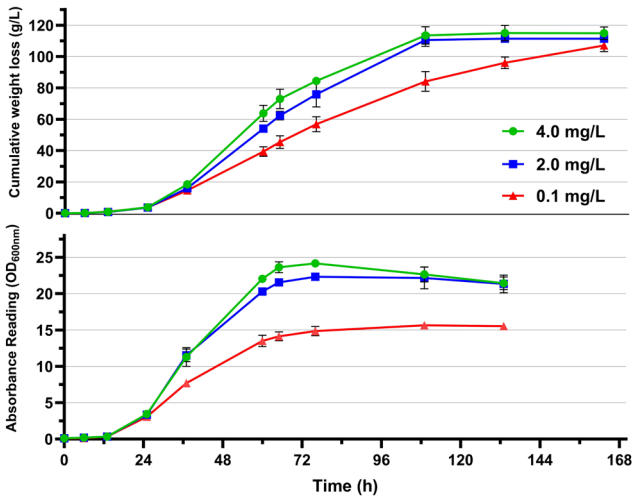


FIGURE 1. Fermentation kinetics (top) and yeast population dynamics (bottom) in synthetic grape juice containing 230 g/L sugar concentration supplemented with three different levels of nicotinic acid. Fermentation kinetics was monitored through the cumulative weight loss due to the loss of CO_2 produced during fermentation. Population dynamics were monitored as an increase in the juice haziness estimated by the absorbance at 600 nm. Each sampling point represents the mean of triplicate values while the error bars depict the standard deviation among the triplicate samples.

Deficient nicotinic acid results in an imbalance in redox cofactors

The concentrations of NAD^+ and NADH redox cofactors were quantified throughout fermentation (Figure 2). Initially, the levels of NAD^+ (the oxidised form) increased until the end of the lag phase (13 h). As the cells transitioned into the exponential phase, NAD^+ levels dropped sharply for each treatment, reflecting the surge in metabolic activity and increased cell growth rate. During this phase, NAD^+ is rapidly reduced to NADH, which accumulates during glycolysis and tricarboxylic acid cycle. The production of ethanol, carbon dioxide, and other metabolites reflects the yeasts' strategy to maintain the balance between oxidised and reduced cofactors. However, despite the yeast's attempts during this peak in metabolic activity, the reduction of NAD^+ is not fully matched by an equivalent reoxidation of NADH[3]. This imbalance results in a decline in both NAD^+ and NADH levels after 24 h. In the 0.1 mg/L deficient NA treatment, a severe redox imbalance was evident, as NAD^+ levels became nearly undetectable after this point. This shortage of NAD^+ due to limited precursors explains the slower fermentation kinetics and reduced biomass production. This redox imbalance impairs key metabolic processes, leading to the sluggish fermentations in *S. cerevisiae* and prematurely stuck fermentations in several non-*Saccharomyces* yeasts due to insufficient NAD^+ regeneration leading to the decline of the yeast[2].

Dynamics of nicotinic acid uptake and metabolism

The uptake and metabolic fate of NA was determined and is depicted as a simplified sketch in Figure 3. The first phase occurs immediately following inoculation, as the yeast adapts to the grape juice environment by gradually increasing its metabolism, mobilising its reserves of vitamin B3 and importing NA as a substrate for NAD^+ synthesis. This is followed by an accumulation phase, where the cells rapidly consume all available NA from the environment. This is achieved before 50 % maximum yeast growth.

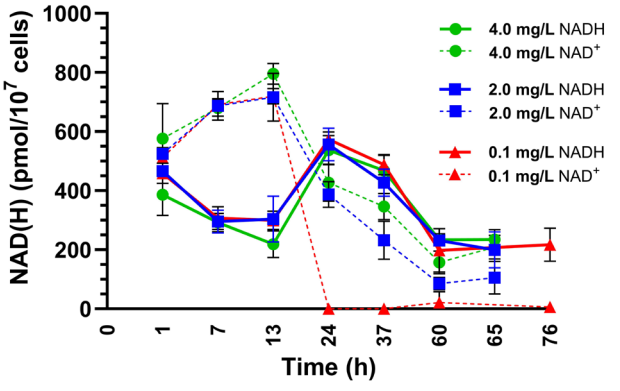


FIGURE 2. The dynamics of intracellular NAD^+ and NADH concentrations in 10^7 cells throughout fermentation in each nicotinic acid treatment. Each sampling point represents the mean of triplicate values while the error bars depict the standard deviation among the triplicate samples.

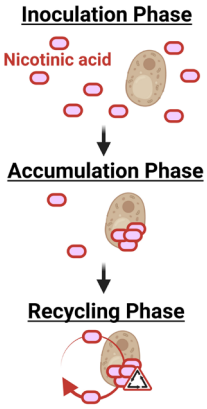


FIGURE 3. The three distinct phases of nicotinic acid uptake in *S. cerevisiae*.

Thereafter, the recycling phase consists of the NAD^+ undergoing conversion into various forms of vitamin B3 in the cell. A limited, yet quantifiable, export of vitamin B3 back into the medium was even observed. Both NA uptake and export are proportional to the initial supplementation level, with higher NA concentrations leading to greater export in later stages.

Deficiency impacts the production of major metabolites

The concentrations of primary metabolites produced across the three treatments were quantified to determine the impact of NA availability (Table 1) in synthetic grape juice. The 0.1 mg/L NA treatment clearly had a profound impact on yeast metabolism, but similar yields of metabolites were observed between the 2.0 mg/L and 4.0 mg/L NA treatments. This indicates the existence of a threshold, whereby beyond this point, further supplementation of NA would not enhance metabolite production or fermentation dynamics. However, deficient levels of NA resulted in significant shifts in metabolite production, as summarised in Figure 4. Specifically, increased production of glycerol, malic acid, and acetoin as compensatory mechanisms for NAD^+ regeneration were observed as well as an increase in succinic acid but a decrease in acetic acid. Ethanol yield remained unaffected by NA concentration, confirming the highly conserved role of ethanol production in yeasts. This underscores the necessity of adequate vitamin B3 levels not only for maintaining redox balance and efficient fermentation but also for stable metabolite profiles.

TABLE 1. Concentrations of metabolites and yeast biomass produced at the end of alcoholic fermentation in synthetic grape juice under fully anaerobic conditions in each nicotinic acid treatment. Each sampling point represents the mean of triplicate values with the standard deviation among the triplicate samples.

Parameter (g/L)	0.1 mg/L	2.0 mg/L	4.0 mg/L
Residual sugars	4.26 ± 1.42 ^{b}	1.21 ± 0.04 ^{a}	1.26 ± 0.03 ^{a}
Biomass*	3.14 ± 0.08 ^{b}	3.53 ± 0.21 ^{a}	3.75 ± 0.31 ^{a}
Ethanol	109.38 ± 0.53 ^{a}	111.05 ± 2.76 ^{a}	110.77 ± 1.08 ^{a}
CO_2	107.07 ± 3.94 ^{a}	111.42 ± 2.46 ^{a}	112.87 ± 4.07 ^{a}
Glycerol	12.75 ± 0.40 ^{a}	9.22 ± 0.56 ^{c}	10.64 ± 0.33 ^{b}
Succinic acid	0.54 ± 0.04 ^{a}	0.24 ± 0.02 ^{b}	0.25 ± 0.01 ^{b}
Acetic acid	0.028 ± 0.01 ^{b}	0.34 ± 0.07 ^{a}	0.38 ± 0.02 ^{a}
Malic acid	2.21 ± 0.24 ^{a}	1.28 ± 0.06 ^{b}	1.38 ± 0.09 ^{b}

*Dry biomass at the end of fermentation.

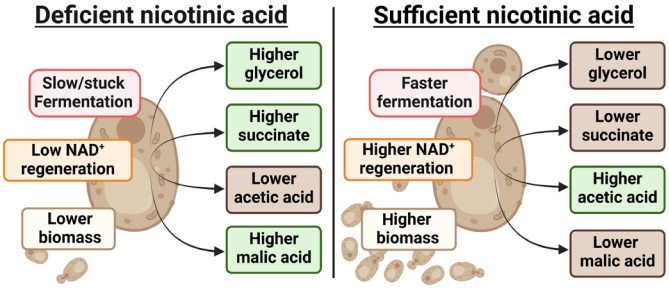


FIGURE 4. The effects of nicotinic acid deficiency and sufficiency on yeast metabolism and fermentation dynamics.

When do deficiencies occur in real grape juice?

Deficiency of vitamin B3 in grape juice is not nearly as common as vitamin B1 (thiamine) deficiency which can occur frequently[1]. However, fungal infections of the grape berry such as from *Botrytis cinerea* deplete nutrients, actively reducing NA concentrations in the grape juice after processing[4]. Early-stage fermentation studies also indicate that thiamine and other B vitamins are quickly assimilated by yeasts[1 5 6], thereby limiting their availability as fermentation progresses. It is also likely that extended pre-fermentative soaking and/or sequential inoculation with non-*Saccharomyces* yeasts further reduces NA levels in the medium, heightening the risks associated with deficiency. While these potential causes of deficiency still need to be confirmed experimentally, supplementation with yeast-derived nutrients containing vitamin B3 is a potentially viable approach to prevent such deficiencies in the above risky scenarios. The controlled addition of oxygen also shows promise, as oxygen allows the *de novo* biosynthesis of vitamin B3 by the yeasts, reducing their reliance on external sources of NA. However, optimising the timing and concentration of oxygen addition, particularly during early to mid-fermentation, is key to ensuring adequate niacin production without compromising fermentation.

Conclusion

This study highlights the role of NA in redox management and metabolic processes in *S. cerevisiae*, particularly for supporting NAD^+ regeneration and efficient fermentation kinetics. Deficient NA levels lead to a severe redox imbalance, as the resulting NAD^+ depletion impairs core metabolic functions, slowing fermentation and reducing biomass. In contrast, excess levels of NA do not enhance fermentation kinetics or metabolite yields beyond those observed under average concentrations, suggesting an optimal threshold for NA supplementation. Additionally, species-specific responses to NA deficiency have been observed, with non-*Saccharomyces* yeasts displaying altered fermentation profiles. Future studies should focus on refining nutrient supplementation strategies and optimised oxygenation for NAD^+ biosynthesis, to ensure smooth fermentations and/or induce favourable metabolic changes. ■

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Sources: *Sourced from the research article: "Nicotinic acid availability impacts redox cofactor metabolism in Saccharomyces cerevisiae during alcoholic fermentation" (FEMS Yeast Research, 24, foae015).*

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