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Production of flavour compounds by wine yeasts is dependent on the management of their intracellular redox balance

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Wine yeasts produce ethanol and a myriad of other metabolites during fermentation that give the final wine its unique organoleptic fingerprint. The complex network of metabolic pathways leading to the production of these flavour compounds is largely dependent on redox enzymatic reactions that make use of the NAD(H) and NADP(H) cofactors. In this work, we investigated the redox cofactor balance and management of two genetically related yeast strains throughout alcoholic fermentation and monitored their production of key metabolites.

Alcoholic fermentation kinetics

The fermentation kinetics of two genetically related strains of *Saccharomyces cerevisiae*, EC1118™ and IONYSwf™[1], were compared in a synthetic grape juice medium containing 230 g/L sugars comprising half glucose and half fructose (Figure 1). IONYSwf™ reportedly produces high amounts of organic acid and glycerol, and low amounts of ethanol and acetic acid[2].

The times corresponding to key fermentation stages were identified. The key stages considered were the following: entry into the exponential yeast growth phase, mid-exponential phase, entry into the stationary phase (which also corresponds to one-third of fermentation completion), two-thirds of fermentation completion and the end of fermentation (signified by sugar concentration below 5 g/L). Both yeast strains yielded typical fermentation profiles and reached dryness, but IONYSwf™ fermented at a slower pace than EC1118™, taking 120 hours to reach dryness whereas the latter required 110 hours.

Dynamics of intracellular NAD(H) concentration throughout fermentation

The intracellular concentrations of the redox cofactor NAD^+, NADH, $NADP^+$ and NADPH were monitored at the key fermentation stages mentioned above. While the concentrations and ratios of $NADP^+$ and NADPH were found to be fairly stable over time (data not shown), those of NAD^+ and NADH were found to vary significantly (Figure 2). Indeed, the total concentration of NAD(H) (*i.e.* the sum of [NAD^+] and [NADH]) was only stable until the mid-exponential phase, after which it decreased drastically in both strains, but with a marked difference. Indeed, the NAD(H) concentration dropped sooner in EC1118™. Similarly, the ratio of NAD^+/NADH dropped sooner in EC1118™ revealing that IONYSwf™ managed to maintain a higher concentration of NAD^+ than EC1118™ after the mid-exponential time point.

It is typically reported in literature that the concentration of NAD(H) and the ratio of NAD^+/NADH are stable throughout fermentation[3,4]. The results of this study reveal that the former assumption on the concentration is incorrect and that the latter on the ratio is only partially correct. These assumptions were mostly based on models established in fed-batch systems when yeasts adopt a respiratory metabolism, a rare occurrence in *S. cerevisiae*, which is subjected to a strong Crabtree effect. Indeed, in this yeast species, sugar concentrations as low as 150 mg/L induce a shift from respiration to fermentation regardless of the presence of oxygen[5]. The data suggest that, as the yeasts lean towards the stationary phase, NAD(H) is progressively converted back to its original substrate nicotinamide.

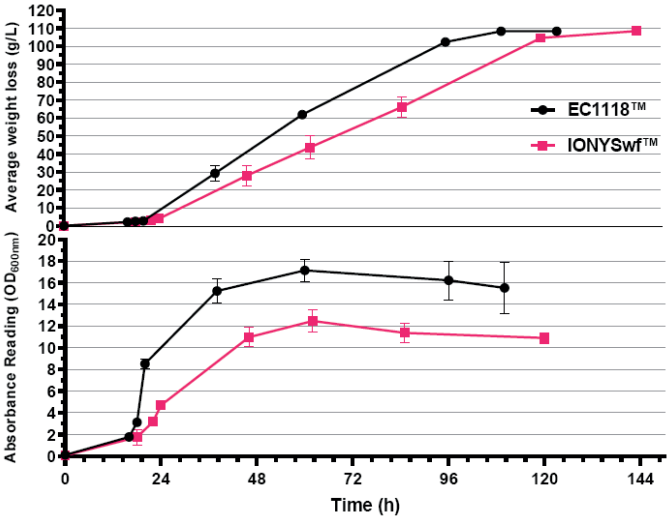


FIGURE 1. Fermentation kinetics and yeast growth of strains EC1118™ and IONYSwf™ in synthetic grape juice containing 230 g/L sugar concentration.

Furthermore, once the stationary phase has been reached, the yeasts struggle to maintain the balance between the two forms of NAD (i.e. NAD^+ and NADH), although some strains seem to manage better than others. The data, therefore, shed new light not only on *S. cerevisiae*'s intracellular redox cofactors management but also on its strain-specific metabolic fluxes. Indeed, the different extent of NAD^+/NADH ratio imbalance suggests differences in the production of metabolites, and ultimately in the flavour profile of the wine made with specific strains.

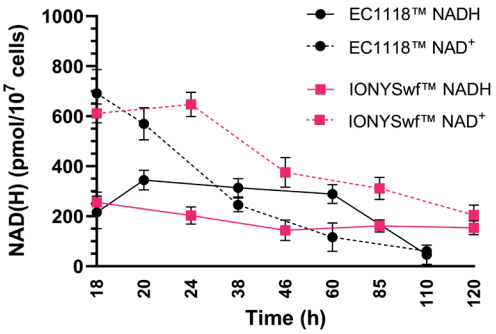


FIGURE 2. Dynamics of intracellular NAD^+ and NADH concentration throughout fermentation in strains EC1118™ and IONYSwf™.

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TABLE 1. Concentrations of metabolites and biomass produced at the end of alcoholic fermentation in synthetic grape juice under fully anaerobic conditions by EC1118™ and IONYSwf™.

	EC1118™	IONYSwf™
Residual sugars (g/L)	0.37 ± 0.03 [a]	2.42 ± 1.93 [a]
Biomass (g/L)	4.56 ± 0.05 [a]	4.43 ± 0.02 [a]
Ethanol (g/L)	104.34 ± 1.97 [a]	100.57 ± 1.49 [b]
CO_2 (g/L)	108.27 ± 0.13 [a]	107.93 ± 0.07 [a]
Glycerol (g/L)	6.78 ± 0.01 [b]	9.83 ± 0.11 [a]
Succinic acid (g/L)	0.46 ± 0.03 [b]	0.92 ± 0.03 [a]
Acetic acid (g/L)	0.66 ± 0.02 [a]	0.45 ± 0.01 [b]
Sum of major higher alcohols* (g/L)	0.24 ± 0.00 [b]	0.32 ± 0.00 [a]
Sum of major acetate esters** (mg/L)	1.32 ± 0.1 [b]	4.75 ± 0.3 [a]

*Sum of Butan-1-ol, Propan-1-ol, 2-methylpropan-1-ol, 3-methylbutan-1-ol and 2-phenylethanol; **Sum of 3-methylbutyl acetate and 2-phenylethyl acetate.

Production of major metabolites

To confirm that strain-specific redox management leads to unique metabolic footprints, the major metabolites produced by EC1118™ and IONYSwf™ were quantified at the end of fermentation (Table 1). To understand the difference in metabolic fluxes between the two strains, the results are shown on a sketch showing the main pathways and end-products of oenological relevance (Figure 3). The results clearly show that IONYSwf™ produces more glycerol, less ethanol, less acetic acid, and more succinic acid as well as more higher alcohols and more acetate esters. While these results confirm those previously reported[2], the knowledge of the peculiar intracellular redox status of this strain compared to EC1118™ allows us to better understand its metabolic activity. Indeed, its increased production of glycerol directly results in lower (although limited in the conditions of this experiment) ethanol production because it reduces sugar availability. From a redox viewpoint, the increase in glycerol production also forces the yeast to produce more compounds that allow it to regenerate more NADH as a compensatory mechanism. These include succinic acid and major aroma compounds such as higher alcohols and acetate esters. These likely explain the higher NAD^+ concentration throughout fermentation.

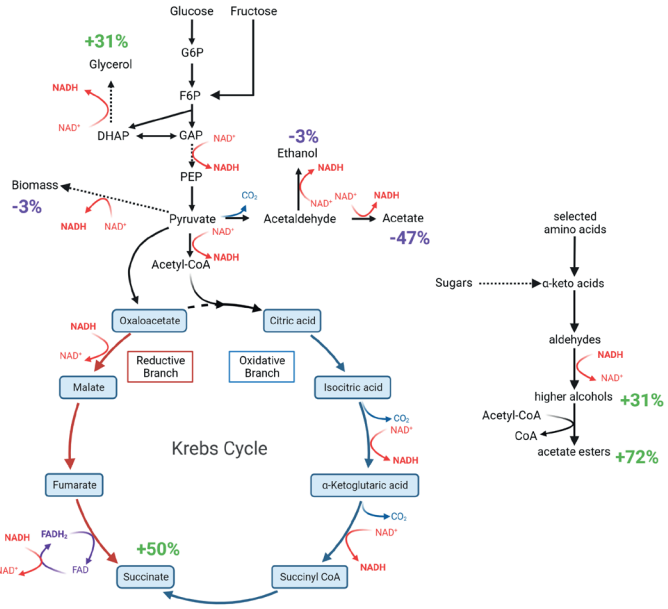


FIGURE 3. Simplified metabolic pathways leading to the formation of main end-products of oenological interest. The reactions involving redox cofactors are depicted in red. The percentage increase or decrease of end-products in IONYSwf™ compared to EC1118™ is indicated in green and purple, respectively, next to the names of the end-products. Doted arrows indicate multiple steps. Abbreviations: G6P: Glycose-6-Phosphate, F6P: Fructose-6-Phosphate, GAP: Glyceraldehyde-3-Phosphate, PEP: Phosphoenolpyruvate, DHAP: Dihydroxyacetone Phosphate.

Conclusion

This study sheds new light on intracellular redox management in the yeast *Saccharomyces cerevisiae*. In particular, it shows that the concentration of NAD(H) decreases over time, which is to be correlated with the decrease of alcoholic fermentation rate after the mid-exponential phase since less cofactors are available to assist with the enzymatic breakdown of sugars. While the intricate connection of primary metabolite production with redox cofactors was known, this study demonstrated clear strain specificity. In the case of IONYSwf™, its inherent high production of glycerol has a metabolic snowball effect. From the data generated in this study, it may be assumed that conditions leading to even higher glycerol production are likely to further reduce ethanol and increase the production of desired by-products such as succinic acid and fermentation aroma compounds. Future research should focus on improving our understanding of the dynamics of NAD(H) production and degradation under various environmental conditions and the link with the production of metabolites of oenological relevance. ■

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Sources : *Sourced from the research article: "Redox cofactor metabolism in Saccharomyces cerevisiae and its impact on the production of alcoholic fermentation end-products" (Food Research International, 2023).*

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