



HAL
open science

Metabolic impact of redox perturbations on the formation of aroma compounds during alcoholic fermentation

Audrey Bloem, Isabelle Sanchez, Sylvie Dequin, Carole Camarasa

► To cite this version:

Audrey Bloem, Isabelle Sanchez, Sylvie Dequin, Carole Camarasa. Metabolic impact of redox perturbations on the formation of aroma compounds during alcoholic fermentation. Oeno 2015, Jun 2015, Bordeaux, France. pp.196-199. hal-01606828

HAL Id: hal-01606828

<https://hal.science/hal-01606828>

Submitted on 2 Jun 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - ShareAlike 4.0 International License

Metabolic impact of redox perturbations on the formation of aroma compounds during alcoholic fermentation

A. BLOEM^{1,2,3*}, I. SANCHEZ^{1,2,3}, S. DEQUIN^{1,2,3}, C. CAMARASA^{1,2,3}

¹INRA, UMR1083 Sciences pour l'Œnologie, Montpellier, France

²SupAgro, UMR1083 Sciences pour l'Œnologie, Montpellier, France

³Université de Montpellier, UMR1083 Sciences pour l'Œnologie, Montpellier, France

Saccharomyces cerevisiae – redox balance – alcoholic fermentation – aroma compounds

Introduction

During alcoholic fermentation, *S. cerevisiae* produces a variety of volatile metabolites, including acetate esters, ethyl fatty acid esters, higher alcohols, volatile fatty acids and volatile sulfur compounds that contribute to the fruity and floral profile of wine. These volatile compounds are formed through a complex and dynamic metabolic process during fermentation and many factors have been reported as controlling their production: grape variety, yeast strain and fermentation conditions (temperature, micronutrients and nitrogen availability in the must) [1].

The redox status of yeast cells could be an additional parameter involved in the qualitative and quantitative modulation of the profile of fermentative compounds. Maintaining the balance between NAD/NADH and NADP/NADPH, which are the main contributors to redox metabolism, is a fundamental requirement to sustain metabolism and growth in *S. cerevisiae*. These cofactors, involved in more than 300 reactions in yeast, are among the most highly connected molecules in metabolic networks [2]. Understanding the links between the cell redox status and the concentrations of volatile compounds in wine has important implications with respect to winemaking parameter such as oxygen and its impact on wine quality. The objective of this study was to gain a better understanding of the effect of the redox balance on the synthesis and regulation of volatile compounds. To this end, the NAD(P)H oxidation was increased under fermentative conditions by the reduction of acetoin to 2,3-butanediol in strains overexpressing native NADH- or engineered NADPH-dependent butanediol dehydrogenase (Bdh) in the presence of acetoin.

Materials and methods

Strains

The strains used were derived from the *S. cerevisiae* haploid strain 59A. Strains NADH-Bdh and NADPH-Bdh were obtained by overexpressing the NADH-dependent 2,3-butanediol dehydrogenase (Bdh1p) or a mutated version of this protein [3] with a NADPH cofactor specificity [4].

Fermentation medium and growth conditions

Batch fermentations were performed in 1-L bioreactors equipped to maintain anaerobiosis, at 24°C, with permanent stirring. Fermentation experiments were carried out in synthetic medium, as described previously [5], containing 100 g L⁻¹ glucose and 100 g L⁻¹ fructose at pH 3.4. Acetoin was added at 200 mM at the beginning of the fermentation. Fermentations were performed at 24°C. CO₂ release was determined by automatic measurement of fermentor weight loss every 20 min.

Metabolite quantification

Glucose, acetate, fructose, glycerol and succinate were analyzed by HPLC using an Aminex HPX-87 ion exchange column. Volatile compounds were determined by GC/MS after dichloromethane extraction procedure with the Siebert GC/MS method [6].

Statistical analysis

Statistical analyses were performed using R software, version 3.1.1 [7]. To obtain a general overview of the final production of volatile compounds, a principal component analysis (PCA) was performed using the FactoMineR package [8]. Each variable was then tested using a one-way analysis of variance (ANOVA) with the conditions (strains overexpressing NADH or NADPH-Bdh with 0 or 200 mM acetoin) as a factor to detect a global effect. In the figures, conditions sharing the same letter are not significantly different at a 0.05 threshold.

Results and discussion

Impact of NADPH and NADH perturbations on aroma compounds

We investigated the impact of an increased demand of cofactors (NADH and NADPH) on aroma compounds whose synthesis involves several aldehyde/alcohol dehydrogenases sensitive to NAD(P)/NAD(P)H ratios. Fermentations with the strains NADH-Bdh and NADPH-Bdh grown without acetoin addition were used as control for the analysis of the incidence on aroma compound formation of NADH and NADPH perturbation, respectively. We found that an increased NADH or NADPH oxidation markedly changed the production of fermentative aroma. Furthermore, the metabolic variations were more important in response to a NADH imbalance compared with a modification of the NADPH demand. A principal component analysis (PCA) was carried out to get a general overview of the incidence of redox modifications on the formation of aroma (Figure 1).

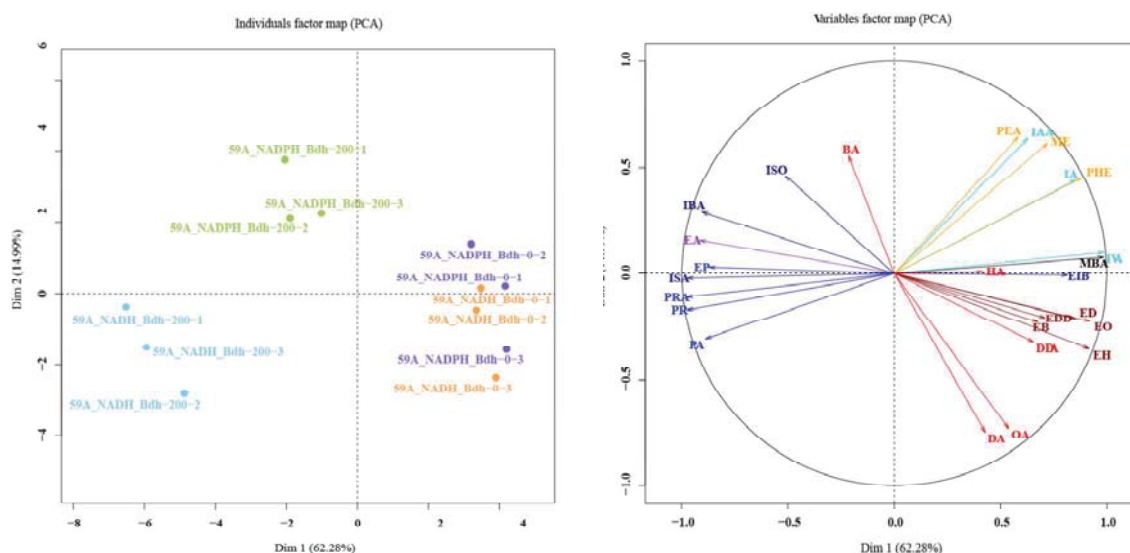


Fig. 1 - Principal component analysis of 59A-NADH-Bdh or 59A-NADPH-Bdh strains supplemented or not with 200 mM acetoin for 26 volatile aroma compounds, based on mean values calculated from 3 replicates. (A) Individual factor map: NADH-Bdh in the presence of 200 mM acetoin (blue) or without acetoin (orange), and for NADPH-Bdh in the presence of 200 mM acetoin (green) or without acetoin (violet); (B) Variable factor map. On the x axis is the percentage of variation explained by principal component 1 (Dim 1; 56.98%); on the y axis is the percentage of variation explained by principal component 2 (Dim 2; 12.84%). ISO: Isobutanol, IBA: Isobutyric acid, BA: butyric acid, EA: ethyl acetate, EP: ethyl propionate, ISA: isobutyl acetate, PRA: propyl acetate, PR: propanol, PA: propionic acid, DA: decanoic acid, OA: octanoic acid, DDA: dodecanoic acid, EH: ethyl hexanoate, EB: ethyl butanoate, ED: ethyl decanoate, EO: ethyl octanoate, EDD: ethyl dodecanoate, EIB: ethyl isobutyrate, MBA: 2-methyl butanoic acid, PEA: phenyl ethyl acetate, ME: methionol, PHE: phenylethanol, IAA: isoamyl acetate, IA: isoamyl alcohol, IVA: isovaleric acid, HA: hexanoic acid.

The final productions of volatile compounds by the strains NADH-Bdh and NADPH-Bdh grown with or without 200 mM acetoin addition were used as quantitative variables. The first two axes accounted for 70% of the total variations and independently separated fermentations with NADH or NADPH imbalance from

the control cultures (NADH-Bdh and NADPH-Bdh without acetoin). The strain NADH-Bdh grown in the presence of acetoin was differentiated along the first axis on the basis of a higher formation of propanol and isobutanol and their derivatives as well as a lower synthesis of medium chain fatty acids and their ethyl esters. This axis also contributed to the discrimination of the NADPH-perturbation modality but to a lesser extent and together with the second axis, mainly defined by the variables isoamyl alcohol, isoamyl acetate, methionol and 2 phenyl ethyl acetate.

Effect of the redox perturbation on the medium chain fatty acid (MCFA) and (MCFA) ethyl ester formation

We then focused on the production of medium chain fatty acids (MCFA) and their ethyl ester derivatives. Contrary to other MCFA, the formation of both butanoic acid (the shortest MCFA) and ethylbutanoate were almost unchanged due to a redox imbalance (Figure 2). These results suggest the existence of other enzymes that can support their synthesis or the existence of several metabolic pathways behind the formation of butyric acid. Indeed, Rollero *et al.* [9] suggested isobutyryl-CoA could be converted to butanoyl-CoA, the precursor of ethyl butanoate.

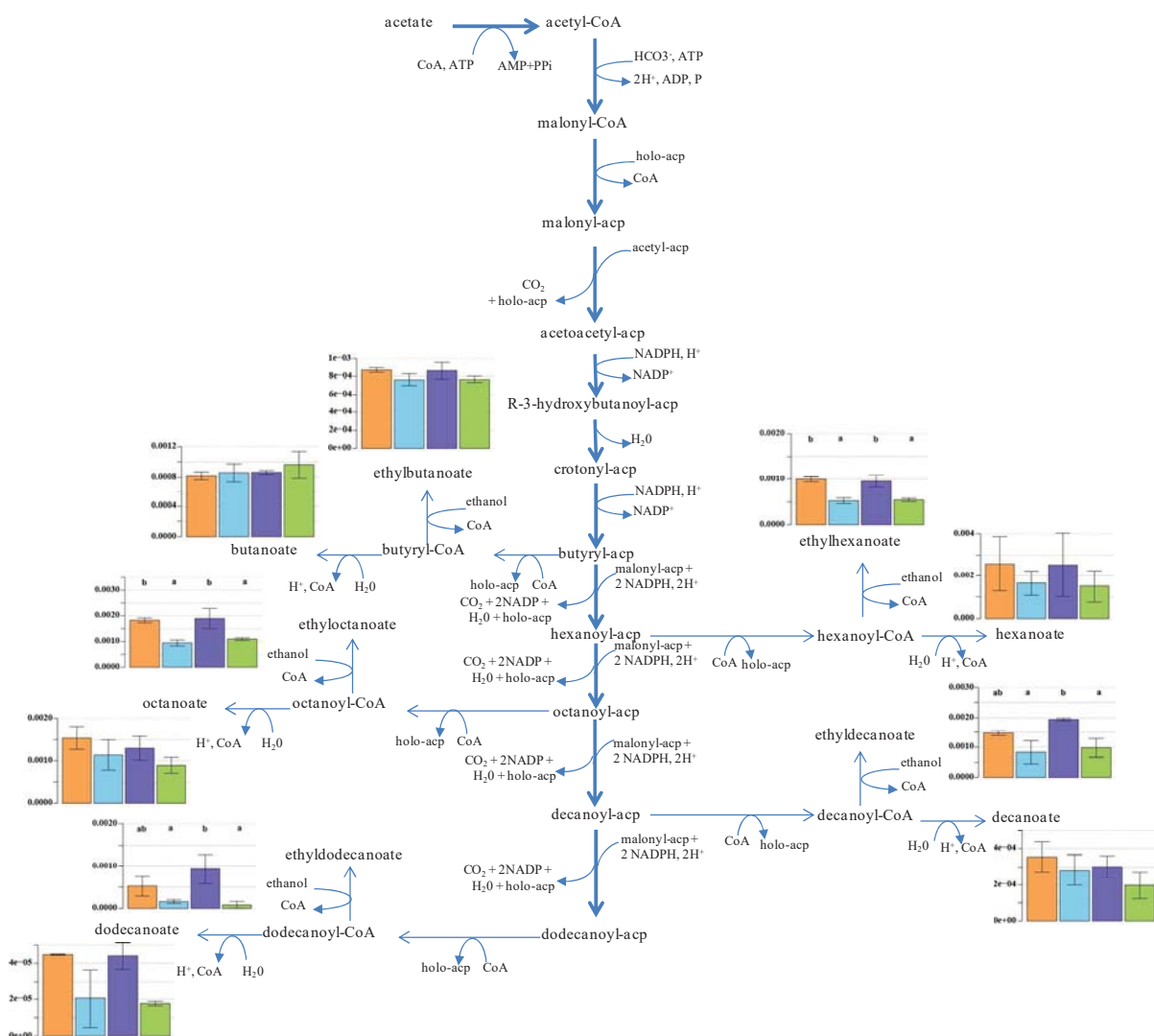


Fig. 2 - Average contents in μM/g DW of medium chain fatty acids and ethyl esters produced by NADH-Bdh and NADPH-Bdh strains in response to redox perturbation (200 mM acetoin). Conditions with the same letter do not differ significantly at Tukey's test (p<0.05). Metabolite concentrations for NADH-Bdh without acetoin (orange) or in the presence of 200 mM acetoin (blue), and for NADPH-Bdh without acetoin (violet) or in the presence of 200 mM acetoin (green).

Further synthesis of MCFA consists in C2-unit elongation of the carbon chain provided by the active malonyl-CoA that requires NADPH. In line with the cofactor requirements for MCFA elongation metabolism, the formation of MCFA (hexanoic, octanoic, decanoic acids) was reduced by 30% on average, irrespective of the nature of the cofactor. In the same way, fatty acid esters deriving from the combination of MCFA with ethanol exhibited similar patterns in relation to redox demand. The NADPH specificity of fatty acid synthase excludes a direct regulation of MCFA production by NADH cofactor availability. One of the most likely explanations for the decrease in MCFA formation with an increased NADH and NADPH demand is a limitation of the intracellular pool of their acetyl-CoA precursor. Moreover, the down-regulation of the gene *EEB1* encoding for the main acyl-CoA-ethanol O-acyltransferase, responsible for ethyl ester formation [10] in response to an increased NAD(P)H oxidation, may also contribute to the reduced production of this family of volatile compounds [11].

Conclusion

These data provide a wide picture of the effects of NADH or NADPH on the aroma network. As esters are central components of the fruity character, a better understanding of the regulation of this network will help to better manage the aroma levels in fermented beverages with improved sensorial quality.

References

1. Swiegers J.H., Bartowski EJ, Henschke PA et al (2005) Yeast and bacterial modulation of wine aroma and flavour. *Aust J Grape Wine Res* **11**: 139-173.
2. Nielsen J (2003) It is all about metabolic fluxes. *J Bacteriol* **185**: 7031-7035.
3. Ehsani M, Fernandez MR, Biosca JA et al (2009) Reversal of coenzyme specificity of 2, 3-butanediol dehydrogenase from *Saccharomyces cerevisiae* and in vivo functional analysis. *Biotechnol Bioeng* **104**: 381-389.
4. Celton M, Goelzer A, Camarasa C et al (2012) A constraint-based model analysis of the metabolic consequences of increased NADPH oxidation in *Saccharomyces cerevisiae*. *Metab Eng* **14**: 366-379.
5. Crépin L, Nidelet T, Sanchez I et al (2012) Sequential use of nitrogen compounds by *Saccharomyces cerevisiae* during wine fermentation: a model based on kinetic and regulation characteristics of nitrogen permeases. *Appl Environ Microbiol* **78**: 8102-8111.
6. Siebert TE, Smyth HE, Capone DL et al (2005) Stable isotope dilution analysis of wine fermentation products by HS-SPME-GC-MS. *Anal Bioanal Chem* **381**: 937-947.
7. R Core Team (2014) *R: a language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>.
8. Husson F, Josse J, Le S et al (2014) *FactoMineR: multivariate exploratory data analysis and data mining with R. R package version 1.27*. <http://CRAN.R-project.org/package=FactoMineR>.
9. Rollero S, Bloem A, Camarasa C et al (2014) Combined effects of nutrients and temperature on the production of fermentative aromas by *Saccharomyces cerevisiae* during wine fermentation. *Appl Microbiol Biotechnol* **99**: 2291-2304.
10. Saerens SM, Verstrepen KJ, van Laere SD et al (2006) The *Saccharomyces cerevisiae* EHT1 and EEB1 genes encode novel enzymes with medium-chain fatty acid ethyl ester synthesis and hydrolysis capacity. *J Biol Chem* **281**: 4446-4456.
11. Celton M, Sanchez I, Goelzer A et al (2012) A comparative transcriptomic, fluxomic and metabolomic analysis of the response of *Saccharomyces cerevisiae* to increases in NADPH oxidation. *BMC Genomics* **13**: 1-14.