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Production of flavour compounds and expression of genes involved in higher alcohol and ester formation during industrial-scale high gravity brewing

Yang He, Lu Chen, Jianjun Dong, Hua Yin

State Key Lab of Biological Fermentation Engineering of Beer, Tsingtao Brewery Co., Ltd., Qingdao, China

Introduction

The ability of lager yeast, *Saccharomyces pastorianus*, to produce a broad range of aroma-active compounds during fermentation is vital to the final taste of beer. Of all the yeast secondary metabolites responsible for the principal flavour of finished beer, higher alcohols and esters are deemed to be a highly significant group of flavour-active metabolites. The lack of control over the production of flavour compounds is a particular problem in modern high gravity brewing, which often leads to disproportionate amounts of higher alcohols and volatile esters.

The hybrid nature of *S. pastorianus*, is characterized by the presence of two divergent sub-genomes, "*S. cerevisiae*-type" (Sc-type), and the "*S. bayanus*-type" (Sb-type) (1). Several genes in the *S. pastorianus* genome are involved in the biosynthesis of flavour compounds (2). However, knowledge regarding the biosynthesis of genes is incomplete. Better knowledge of flavour development and expression profiles of the genes involved, in the context of fermentation progression, is an important aspect for the brewing industry to regulate the concentrations of sensory metabolites in beer.

Project Aims

The aims of the project were: (a) to compare the flavour production of higher alcohols and volatile esters under standard conditions and high gravity fermentation; (b) to find out how the expression levels of genes involved in higher alcohols and volatile esters change with regards to wort gravity; and (c) to differentiate and compare the expression levels of the Sc-type and corresponding orthologous Sb-type genes present in lager yeast.

Methods

- All fermentations were carried out in 2000 L fermenters.
- Experiments were performed using an industrial lager brewing strain, *S. pastorianus* TT-21, obtained from a Tsingtao brewery yeast storage vessel.
- Gravity and alcohol content were measured with a DMA 4500 density analyser and Alcolyser Plus.
- Gene expression was applied at around 40% apparent degree of fermentation in the GenomeLab™ GeXP Genetic Analysis System (3).
- Higher alcohols and esters concentrations were measured by headspace gas chromatography (4).

Results and Discussion

Fermentation characteristics

- The final gravity was 2.7 ° P for both 13° P and 16° P fermentations at 157 h .
- A peak in yeast biomass in suspension was observed at 86 h in 13° P fermentation, after which a decrease was noted, owing to flocculation and sedimentation. For 16° P fermentation, the yeast flocculation was occurred ahead of time compared to that of 13° P fermentation.

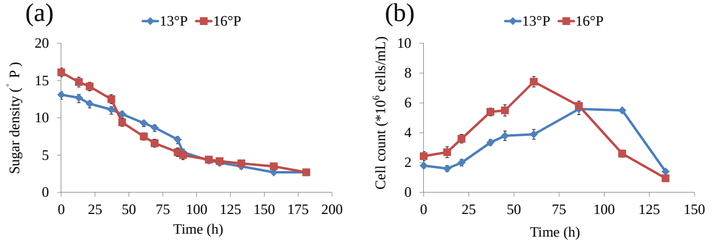

Fig. 1 Fermentation monitored by changes in (a) sugar density and (b) yeast cell counts in suspension during 13° P and 16° P fermentations of *S. pastorianus* TT-21.

Flavour compounds production

- High gravity brewing elevated both higher alcohols and volatile esters production, with significant increase in ethyl acetate level.
- The increment of total higher alcohols was less than that of volatile esters, which leads to a distinct decrease in ratio of total higher alcohol to total ester.

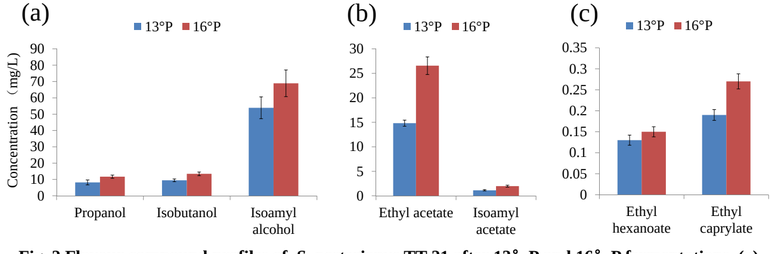

Fig. 2 Flavour compound profiles of *S. pastorianus* TT-21 after 13° P and 16° P fermentations. (a) Higher alcohols (b) Acetate esters and (c) Ethyl esters.

Table 1. Total higher alcohol and total ester produced after 13° P and 16° P fermentations

	Total higher alcohol (mg/L)	Total ester (mg/L)	Ratio of total higher alcohol to ester
13°P	71.6	16.27	4.40
16°P	94.08	28.96	3.25
Percent of increment	31%	78%	/

Transcription of genes involved in higher alcohol and volatile ester synthesis

- The transcription level of all the higher alcohols and volatile esters biosynthesis related genes were enhanced by high gravity brewing, except the expression of *BAT2* (Sc- & Sb-) which were significantly decreased in 16° P fermentation.
- Orthologous gene sets were differentially expressed. No expression of *ATF1*-Sc, *ATF2*-Sc and *IAH1*-Sc were observed during 13° P fermentation.
- Changes in flavour compounds production in high gravity brewing appears to be due at least in part to transcription level of genes involved in the flavour biosynthesis process.

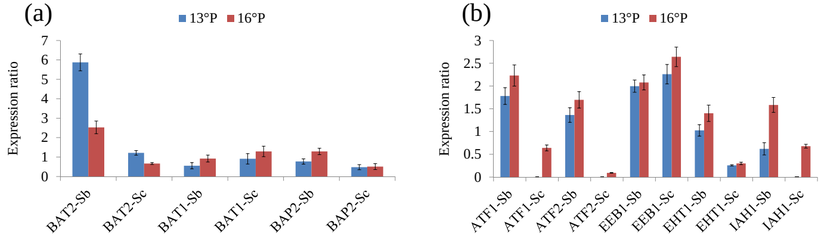

Fig. 3 Expression profiles of *S. cerevisiae* (Sc) and *S. bayanus* (Sb) orthologues genes responsible for (a) higher alcohol and (b) ester synthesis *S. pastorianus* TT-21.

Conclusions

- Wort gravity greatly affected the formation of flavour compounds. High gravity fermentation elevated levels of esters production concurrent with a much less marked degree of higher alcohols stimulation , making it difficult to match to flavour of beers produced from normal gravity wort.
- High gravity wort stimulated transcription of genes involved into higher alcohol and ester synthesis, except individual genes responsible for higher alcohol synthesis pathway.

References

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