Modulation of polyphenols in β-farnesene fed-batch fermentation

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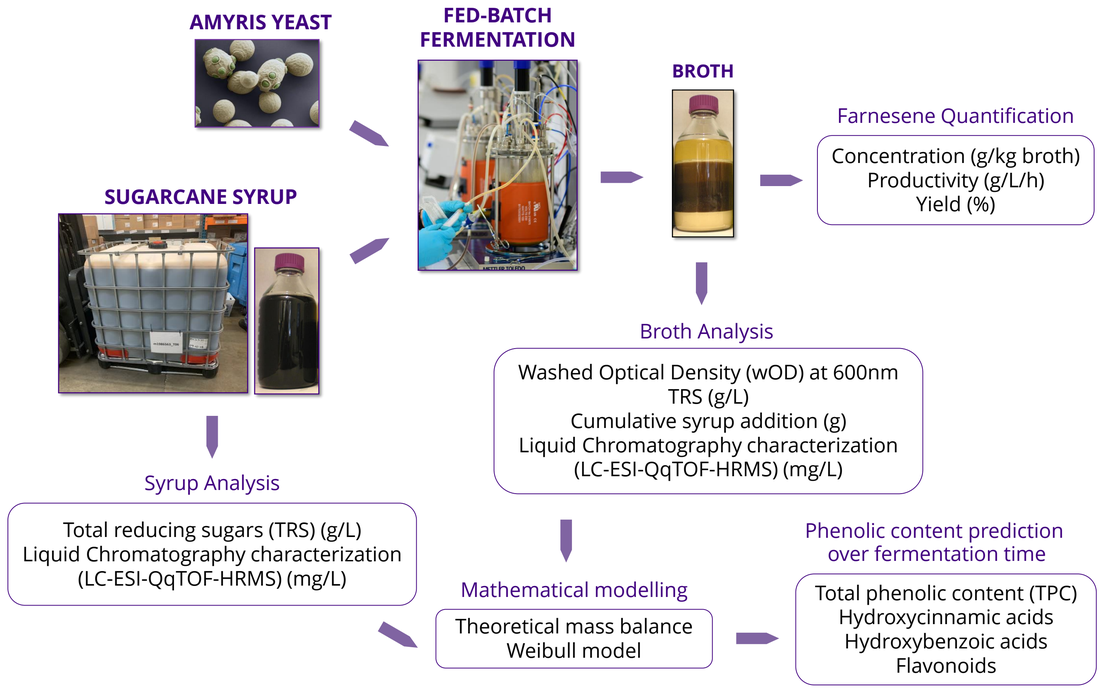
Introduction

- β-Farnesene, which can be in a variety of consumer and industrial products, is currently produced by genetically modified *Saccharomyces cerevisiae* using sugarcane syrup [1,2].
- Sugarcane syrup contains a complex mixture of phenolic compounds derived from the sugarcane plant, which may exert antimicrobial and/or antioxidant effects, and thus influence the fermentation process [3].
- Characterization and modelling of the phenolic content variation during laboratory fermentations can help to predict the phenolic content variation during the farnesene industrial process [4].
- The aim of this work was to modulate the transfer of phenolic compounds from sugarcane syrup to the culture broth over 13 days of β-farnesene fed-batch fermentation with an Amyris engineered yeast, reproducing the industrial process conditions in 2 L bioreactors.

Methodology



Results and Discussion

SUGARCANE SYRUP CHARACTERIZATION

Table 1 – Characterization (LC-ESI-UHR-QqTOF-MS) of sugarcane syrup used in the fed-batch fermentations.

Parameter	Average ± STD of 2 batches
TRS (% - w/w)	58.17 ± 0.20
TPC (mg/L)	50.66 ± 4.16
Hydroxybenzoic acids (mg/L)	14.66 ± 0.48
Hydroxycinnamic acids (mg/L)	25.21 ± 3.03
Flavonoids (mg/L)	10.80 ± 1.62

- TPC was 50.66 mg/L, represented by 3 classes: hydroxybenzoic acids (29.2 %), hydroxycinnamic acids (49.6 %) and flavonoids (21.2 %) (Table 1).
- Among the 49 identified compounds, the most abundant one was trans-3-feruloylquinic acid, a hydroxycinnamic acid, at 7.22 mg/L.

PHENOLIC COMPOUNDS DURING FED-BATCH FERMENTATION

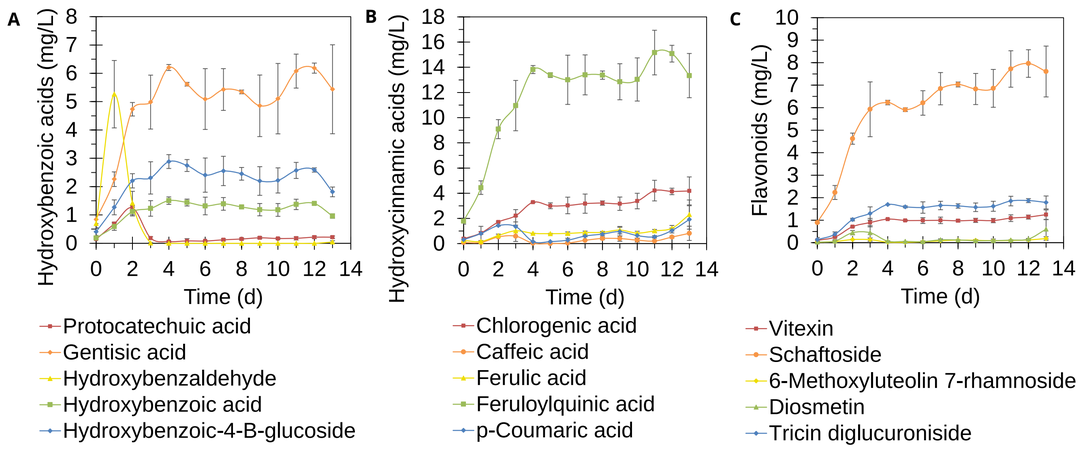


Figure 1 – Hydroxybenzoic acids (A), hydroxycinnamic acids (B) and flavonoids (C) measured in LC-ESI-UHR-QqTOF-MS during the incubation time of fed-batch fermentations of farnesene producing *S. cerevisiae*. Plotted lines represent the average concentration of 4 reactor fermentations and error bars are standard deviations.

- The concentration of most compounds increased until day 2 and then remained constant (Figure 1), reaching levels in the range of those found in the syrup.
- Some compounds (hydroxybenzaldehyde, protocatechuic, caffeic, ferulic and *p*-coumaric acids) decreased after the 2nd day of fermentation, suggesting possible degradation, or yeast metabolization [5,6,7].

MODULATION OF PHENOLIC COMPOUND ACCUMULATION IN FED-BATCH FERMENTATION

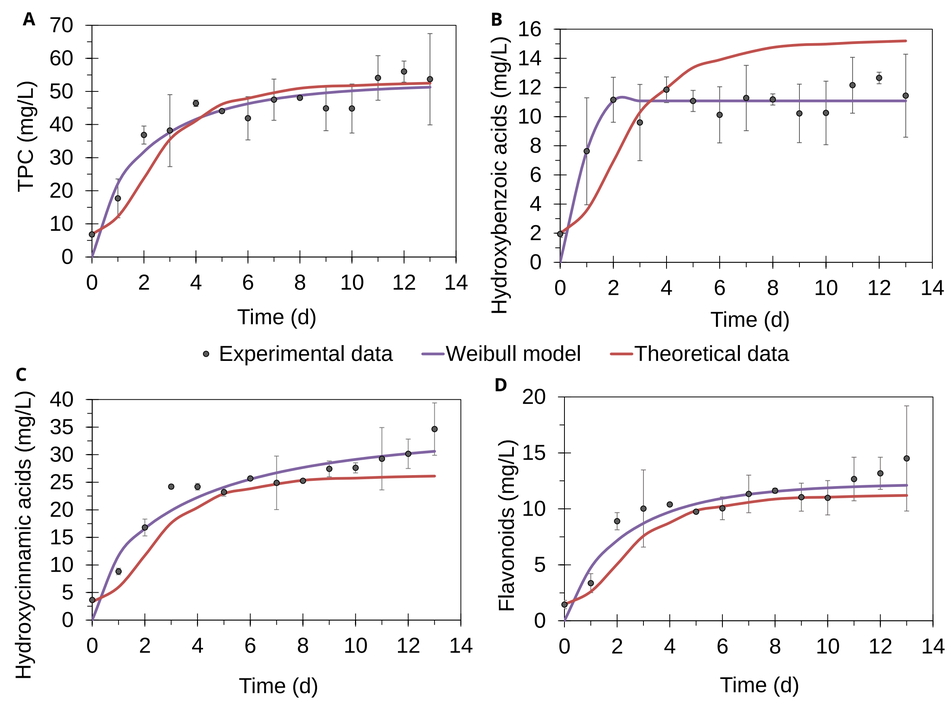


Figure 2 – TPC (A), hydroxybenzoic acids (B), hydroxycinnamic acids (C) and flavonoids (D) measured in LC-ESI-UHR-QqTOF-MS during the incubation time of fed-batch fermentations of farnesene producing *S. cerevisiae*. Plotted dots represent the average of 4 reactor fermentations, error bars are standard deviations and plotted lines correspond to the fit of the Weibull model and the mass balance.

- The Weibull model and the theoretical mass balance successfully described the phenolic content during the fermentation (Figure 2).
- The mass balance presented a coefficient of determination (R^2) of 0.88 for TPC and a root square mean error (RMSE) of 1.54. On the other hand, the Weibull model presented an R^2 = 0.93 and an RMSE of 1.16. Therefore, the Weibull model presented a fit closer to the experimental data.
- The mass balance data did not explain the hydroxybenzoic acids behavior by presenting a low fit (R^2 = 0.66) to the experimental data of this class.

Conclusions

- In sugarcane syrup, the most representative phenolic compound was the trans-3-feruloylquinic acid, that belongs to the hydroxycinnamic acids class.
- The decrease observed for hydroxybenzaldehyde, protocatechuic, caffeic, ferulic and *p*-coumaric acids might be explained by their degradation or metabolization by the yeast during the fermentation.
- The phenolic accumulation inside the bioreactors was successfully described by both mathematical calculations, but the Weibull model presented a better fit.
- This work provided a tool to predict the evolution of phenolic compounds in industrial fed-batch fermentations when sugarcane syrup is used as feedstock.

References

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