Phenolic compound modulation in yeast fed-batch fermentation using sugarcane syrup as feedstock

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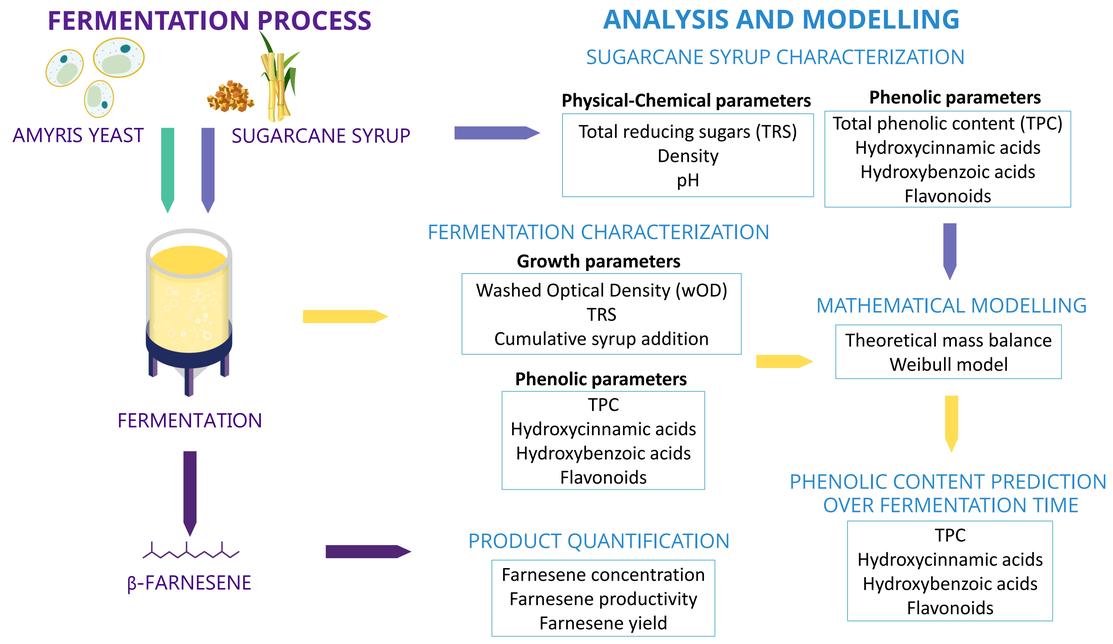
Introduction

- In **industrial fermentations**, plant biomass, such as **sugarcane syrup**, is used to provide the carbon source for the yeast (*Saccharomyces cerevisiae*) to grow and biosynthesize the desired value-added compounds, such as β-farnesene [1,2].
- Sugarcane syrup contains several **phenolic compounds** that may influence the fermentation process, because they exert **antimicrobial** and/or **antioxidant activities** [3].
- **Characterization** and **modelling** of the phenolic compounds during laboratory fermentations can help to predict the phenolic content during the farnesene industrial process [4].
- This work aimed to **characterize the phenolic content of sugarcane syrup** used for the industrial production of β-farnesene in the fermentation process of Amyris Inc.
- Furthermore, the objective was also to characterize and **model the phenolic content** in 2 L laboratory bioreactor fermentations simulating the industrial process (13 days of farnesene fed-batch fermentation with an Amyris engineered strain).

Methodology



Results and Discussion

SUGARCANE SYRUP CHARACTERIZATION

- Characterization of two batches of sugarcane syrup, used in industrial fermentations, was performed (Table 1). **Differences in phenolic compounds** were found between both batches, associated with **seasonality** of the raw material.

Table 1 – Characterization of sugarcane syrup used in industrial fermentations, using LC-ESI-UHR-QqTOF-MS. Different letters inside parenthesis represent significant difference between batches in the same parameter.

Parameter	Batch A	Batch B	Average
TRS weight (%)	57.68 ± 0.28 (a)	58.66 ± 0.04 (b)	58.17 ± 0.20
Density (g/mL)	1.28 ± 0.00 (a)	1.30 ± 0.00 (b)	1.29 ± 0.01
pH	5.93 ± 0.01 (a)	5.75 ± 0.03 (b)	5.84 ± 0.09
TPC (mg/L)	54.77 ± 0.57 (a)	46.55 ± 0.74 (b)	50.66 ± 4.16
Hydroxybenzoic acids (mg/L)	14.20 ± 0.15 (a)	15.12 ± 0.14 (b)	14.66 ± 0.48
Hydroxycinnamic acids (mg/L)	28.22 ± 0.07 (a)	22.19 ± 0.35 (b)	25.21 ± 3.03
Flavonoids (mg/L)	12.35 ± 0.88 (a)	9.24 ± 0.25 (b)	10.80 ± 1.62

- Three chemical classes of phenolic compounds were detected in syrup, namely **hydroxybenzoic acids**, **hydroxycinnamic acids** and **flavonoids**. Hydroxycinnamic acids was the most common class, representing **49.6 % of TPC**. Hydroxybenzoic acids represented 29.2 % and flavonoids 21.2 %.
- The compound with the highest concentration was ***trans*-3-feruloylquinic acid**, a hydroxycinnamic acid, at 7.22 mg/L. The hydroxybenzoic acid found at higher level was **gentisic acid** at 5.39 mg/L and the flavonoid was **isoschaftoside**, at 4.77 mg/L. Most of the identified compounds have been reported to exert antimicrobial and/or antioxidant activity.

PHENOLIC COMPOUNDS DURING FED-BATCH FERMENTATION

- Phenolic compounds were quantified during the fed-batch fermentation (Figure 1). Lowest TPC was found upon inoculation of the bioreactors and maximum was present in the end of the fermentation.
- Hydroxycinnamic acids and flavonoids concentration **increased over time**, while hydroxybenzoic acids concentration **stabilized after 48 h**. In the literature, hydroxybenzoic acid, protocatechuic acid, hydroxybenzaldehyde, caffeic acid, ferulic acid and *p*-coumaric acid were found to be **metabolized by *S. cerevisiae***, corroborating their decrease in the fermentation broth [5,6,7].

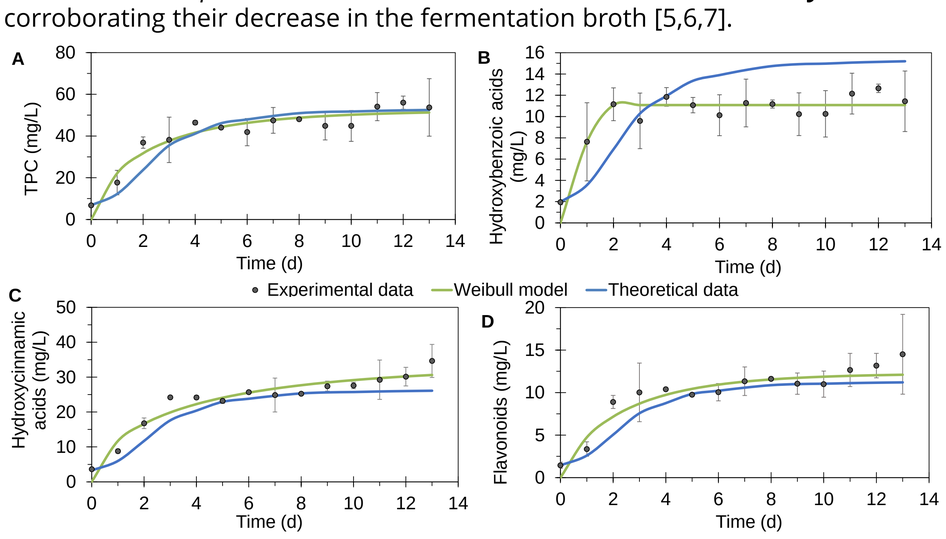


Figure 1 – TPC (A), hydroxybenzoic acids (B), hydroxycinnamic acids (C) and flavonoids (D) measured in LC-ESI-UHR-QqTOF-MS during the incubation time of fed-batch fermentations of farnesene producing *S. cerevisiae*. Plotted dots represent the average of 4 reactor fermentations, error bars are standard deviations and plotted lines correspond to the fit of the Weibull model and the theoretical mass balance data.

- Two mathematical calculations were used to predict the phenolic content during the fermentation. The **theoretical data**, which used a mass balance approach, presented a coefficient of determination **(R^2) of 0.88** and a root square mean error **(RMSE) of 1.54**. On the other hand, the **Weibull model** presented an **R^2 = 0.95 and an RMSE of 1.16**.
- **Both models** successfully described the phenolic content during fermentation, but the **Weibull model** presented a fit **closer to the experimental data**.
- Theoretical mass balance data did not explain the hydroxybenzoic acids behavior by presenting a **low fit (R2 = 0.66)** to the experimental data.

Conclusions

- In sugarcane syrup, the most prevalent phenolic class was found to be **hydroxycinnamic acids** and ***trans*-3-feruloylquinic acid** was the most representative compound.
- 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 way to **predict the phenolic content** inside the culture broth in industrial fermentations.

References

[1] Carsanba, et al. (2021) Pharmaceuticals, 14(4), 295 **[2]** Meadows, et al. (2016) Nature 537, 694–697 **[3]** Adeboye, et al. (2014) AMB Express, 4, 46. **[4]** Hill, et al. (2020) Journal of Industrial Microbiology & Biotechnology, 47, 965–975. **[5]** Gu, et al. (2015) Biotechnology and Bioengineering, 112(9), 1770-1782 **[6]** Fletcher, et al. (2019) Metabolic Engineering, 52, 98–109 **[7]** Goodey, et al., (1982) Journal of General Microbiology, 128(261) 5-2620.

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