Biotechnological production of 2-phenylethanol from agro-industrial wastes for foods and flavouring applications

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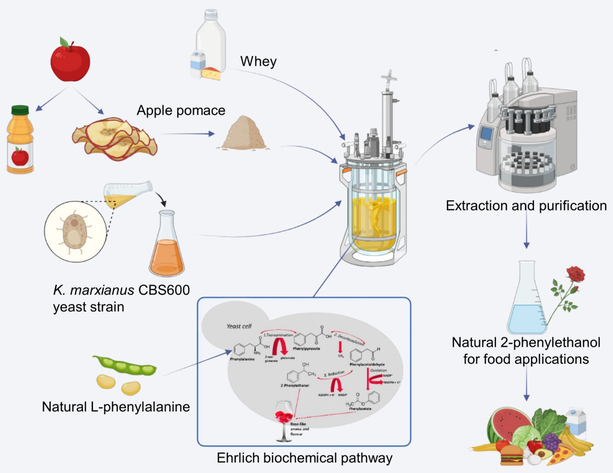
INTRODUCTION

- 2-phenylethanol (2-PE) is a valuable **aroma and flavor compound** with distinct rose odor, widely used in cosmetic and food industries.[1,2]
- Current methods of 2-PE production rely on the use of toxic reagents for chemical synthesis processes, or on its expensive and season-dependent extraction from flowers.[2]

Production of 2-PE via **microbial fermentation** using **agro-industrial wastes** offers a more sustainable and cost-effective solution.

- ✔ **Low-cost substrates**
- ✔ **Food-grade** wild yeast strain (with QPS and GRAS status)
- ✔ In situ product recovery (ISPR) technique with a **green solvent**
- ✔ **Natural 2-PE** approved for food applications



METHODS

Screening in flasks
- Evaluation of 2-PE production from 3 wastes: acid whey, sweet whey, apple pomace
- Acid whey supplementation with yeast extract and minerals
- Experiments in 250 mL baffled shake flasks

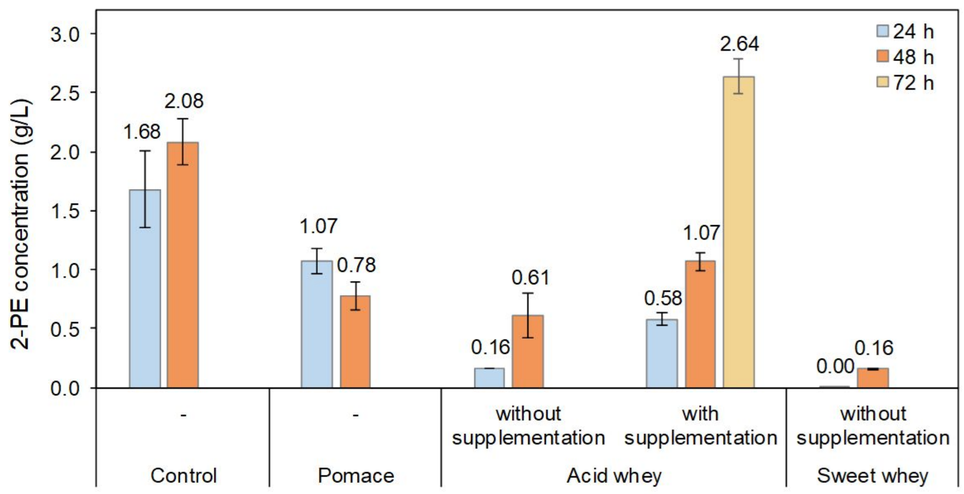
Production in bioreactors
- Fermentation of the selected waste stream in 1 L bioreactors
- Evaluation of the effect of ISPR
  - membrane-based solvent extraction using oleyl alcohol
  - 2 extractions of 60 min: at 24 h and 48 h of fermentation

Techno-economic and environmental assessment
- Process design and simulation with SuperPro Designer v10
- Estimation of capital investment, operational costs, and financial indicators
- Assessment of environmental impacts using OpenLCA

OBJECTIVES: Waste stream selection → Scaling up to a bench bioreactor Demonstration of technical feasibility → Demonstration of economic viability and environmental performance

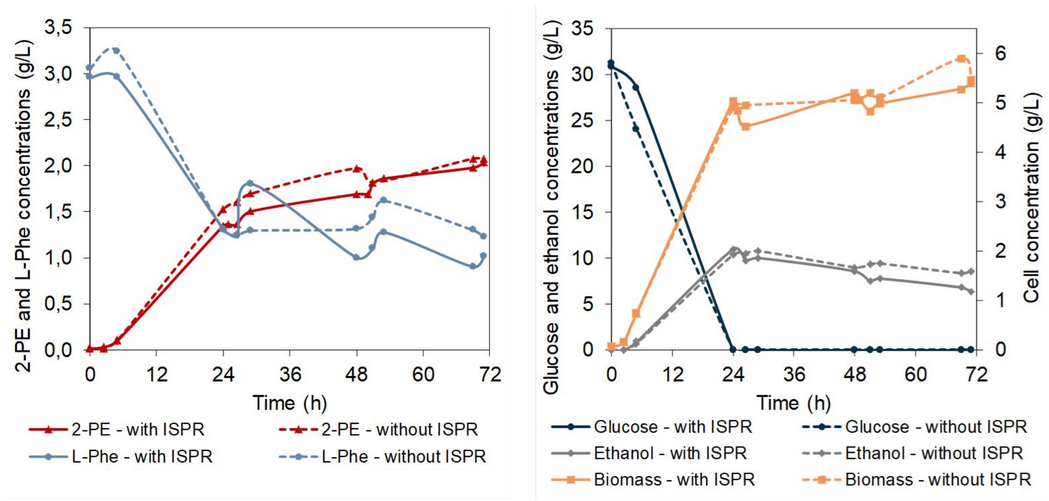
RESULTS

Screening of waste streams



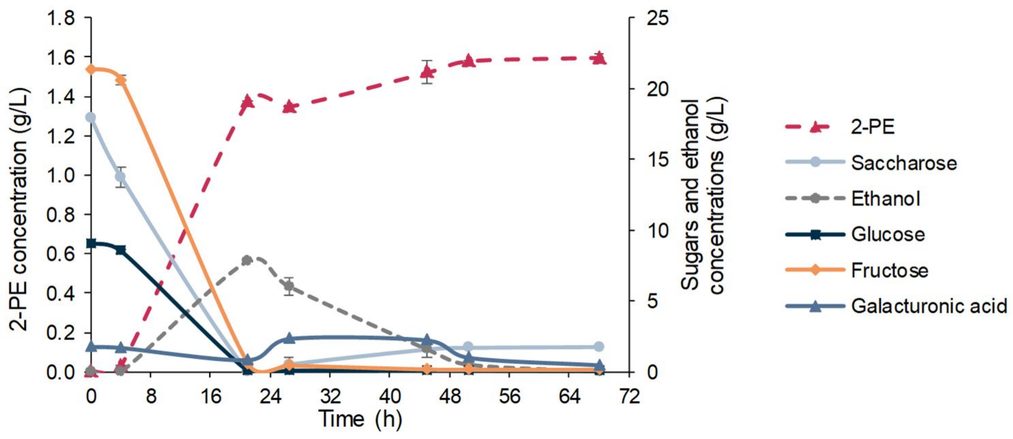
- **Apple pomace** fermentation resulted in the **highest 2-PE productivity** in shake flasks, reaching a concentration of 1.07 ± 0.11 g/L in 24 h.
- Fermentation of **acid whey** was significantly improved **with yeast extract and minerals supplementation**, achieving similar substrate yield (151.5 $mg_{2\text{-}PE}/g_C$) compared to control (170.7 $mg_{2\text{-}PE}/g_C$) and apple pomace (140.5 $mg_{2\text{-}PE}/g_C$).

Fermentation with defined medium in bioreactors coupled to ISPR



- Production yields were similar in the control (0.379 $g_{2\text{-}PE}/g_{cells}$) and ISPR-coupled (0.377 $g_{2\text{-}PE}/g_{cells}$) bioreactors, demonstrating that **cell growth was not affected by ISPR**.
- 2-PE volumetric productivity was 0.029 g/(L.h) in both bioreactors after 70 h, while the maximum productivity of 0.056 g/(L.h) was observed at 24 h.

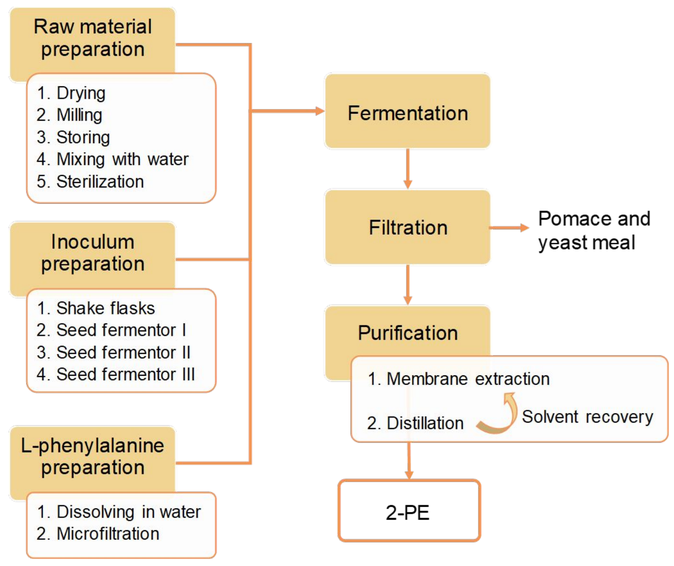
Fermentation of apple pomace in bioreactors



- 2-PE production from apple pomace fermentation was validated in bioreactors, achieving 1.581 ± 0.003 g/L in 50 h.
- Maximum productivity of 0.066 g/(L.h) was achieved with 1.38 g/L in 21 h.

Techno-economic assessment

A biorefinery was designed to produce **185 t/year of 2-PE**, equivalent to ~2% of the global 2-PE market volume. The selling price of 2-PE was set at **350 US$/kg.**



Equipment purchase cost US$21.3 million
Capital investment US$136 million
Unit production cost 245 US$/kg
Net present value US$73 million
Internal rate of return 13.8%

- With a unit production cost of 245 US$/kg of 2-PE and internal rate of return of 13.8%, the project displays a **high profitability potential**.
- 2-PE fermentation is an economically viable process with a **competitive price** compared to the natural 2-PE extracted from flowers, which is priced at 300–1000 US$/kg [2].

Life-cycle assessment

Environmental impacts for the production of 1 kg of 2-PE through microbial fermentation ( ) vs. impacts for cultivation of 116,000 roses* ( )

Climate Change	80.3 kg CO_2 eq	[125,050] kg CO_2 eq
Eutrophication	0.58 mol N eq	[1054.8] mol N eq
Water Consumption	8634.2 m³	[6958.8] m³
Land Use	157.6 Pt	[210,733] Pt
Acidification	0.057 mol H+ eq	[273.3] mol H+ eq
Resource Use, fossils	1902 MJ	[2,153,650] MJ

- Environmental impacts for 2-PE production via microbial fermentation of apple pomace are drastically lower than those reported for flowers cultivation, except for water consumption.

* **116,000 roses**, or around **8 tons of rose petals,** are required for producing 1 kg of 2-PE based on the average 2-PE content in roses of 125 mg/kg.[3] Environmental impacts shown for roses cultivation are taken from Helmes et al. (2021) for roses produced in a Dutch greenhouse.[4]

CONCLUSIONS

- Apple pomace fermentation resulted in the highest 2-PE productivity in shake flasks, reaching a concentration of 1.07 g/L in 24 h, while its upscaling to a 1 L bioreactor led to 1.58 g/L in 50 h.
- Supplementation of whey with yeast extract and minerals significantly improved 2-PE production.
- The use of in sit*u* product recovery (ISPR) in bioreactors with defined medium allowed an efficient 2-PE extraction without negative impacts on 2-PE production or cell growth and viability. This technique shows promise for 2-PE recovery from apple pomace fermentation broths.
- 2-PE production by fermentation is a **sustainable and economically viable process** with competitive price and lower environmental impacts compared to the natural 2-PE extracted from flowers.

References: [1] Martínez-Avila et al., *Appl. Microbiol. Biotechnol.* **102**, 9991–10004 (2018). [2] Mitri *et al.*, *Foods*. **11**, 109 (2022). [3] Roccia et al., *Plant Physiology*, **179**, 1064–1079 (2019). [4] Helmes et al., *Environmental footprint of roses: representative product study*. Wageningen Economic Research, Report 2021-018.

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