Rapid characterization of the chemical profiles of cocoa beans fermented with anti-fungal co-cultures

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1 Introduction

- Functional fermentation co-cultures enhance cocoa beans quality by **preventing fungal contaminations** [1, 5, 6]
- Rapid Evaporative Ionization Mass Spectrometry (REIMS) allows the **characterization** of the untargeted **metabolite profiles of beans** [2, 3]
- The study aims to develop an innovative approach to **facilitate the selection of the microorganisms** with antifungal activity based the metabolite profile of the beans

2 Material

Cocoa beans fermented with and without antifungal co-cultures according to Romanens et al. [4, 5] with modifications:

- **Production:** Micro lab-fermentations conducted inoculating fresh beans with LAB-yeast co-cultures (see table)

Tab 1. Fermentation co-cultures applied

Fermentation co-cultures [5]	Antifungal activity [5]
Lb. fermentum 223 + *S. cerevisiae* H290	Strong
Lb. fermentum 223 + *H. opuntia H17*	
Lb. fermentum M017 + *S. cerevisiae* H290	
Lb. fermentum M017 + *H. opuntia* H17	
Lb. fermentum 193 + *S. cerevisiae* H354	None
Control	

- **Validation:** Antifungal activity of the co-cultures validated by adding *A. flavus* S075 into the inoculated beans

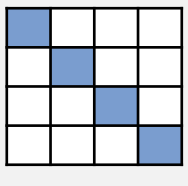
Fig 1. Validation of the antifungal activity of the co-cultures

3 Method Overview

Acquisition of the metabolite fingerprints of the beans using REIMS in negative ion mode

Data processing: peak and lock mass detection, binning, normalization, background subtraction and classification

Modeling: Multivariate data analysis



Validation of the model using a 'leave 20% out' test

4 Results and Discussion

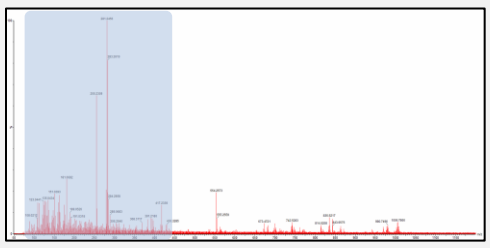


Fig 2. Typical mass spectra of metabolite fingerprint of cocoa beans obtained by REIMS

The region between **50 and 400 m/z** shows:
- The **most abondance** of ionized molecules
- Molecules **differentiating** the most the mass spectra

- Cocoa beans fermented with antifungal co-cultures **can be differentiated** from the others
- This model allows the recognition of the metabolite fingerprints of the beans with a **correctness of 95.29%**
- Three **main chemical markers**: 281.25 m/z, 283.25 m/z and 255.25m/z

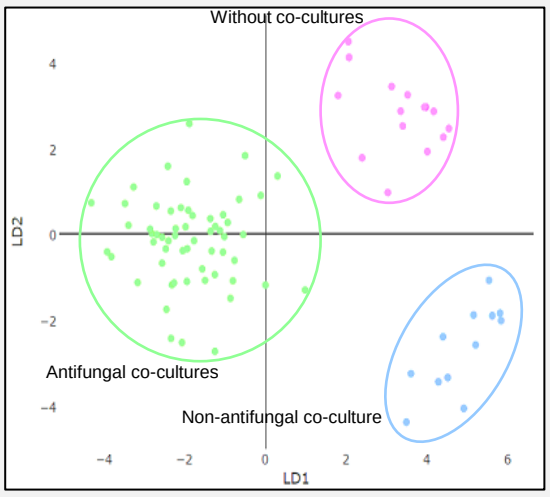


Fig 3. PCA-LDA model of the metabolite fingerprints of the cocoa beans fermented with antifungal co-cultures (green), with a non-antifungal co-culture (blue) and without co-culture (pink)

5 Conclusion

- The rapid fingerprinting method combined with advance data analysis is a **promising approach to select microbial strains** with antifungal activity
- REIMS technic allows **the rapid and real-time characterization** of the metabolite profiles of cocoa beans
- Cocoa beans fermented with antifungal co-cultures can be **differentiated** thanks to PCA-LDA model based on the metabolite profiles
- This study contributes to the sustainable cocoa production by **improving bio-preservation** and **reducing waste** from microbial spoilage benefiting farmers and retailers.

References

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