

## Predicting the metabolome of synthetic microbial communities to reduce anti-nutritional factors and off-flavours in pulses

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### **Aim:**

The demand for plant-based, protein-rich, and sustainably produced food sources is growing. European pulses such as faba beans and yellow peas are nutritious, cultivatable in temperate climates, and possess desirable functional properties for application in meat and fish analogues. However, their use is limited by the presence of anti-nutritional factors (ANFs), including protease inhibitors and phytic acid, and off-flavours such as hexanal. Both ANFs and off-flavours can be modified by chemical, physical, or biological treatment such as fermentation. Since the latter shows the greatest potential, a systematic, knowledge-based, design of synthetic microbial communities offers a powerful approach to optimize fermentation with the aim to reduce ANFs and off-flavours and to advance our understanding of microbial interactions in pulse fermentation.

### **Method:**

We investigated all possible microbial communities composed of selected strains commonly used in food fermentation: *Lactococcus lactis*, *Leuconostoc mesenteroides*, *Propionibacterium freudenreichii* subsp. *freudenreichii*, *Acetobacter aceti* subsp. *aceti*, and *Saccharomyces cerevisiae*. After fermentation, volatile compounds were measured making use of gas chromatography, while non-volatile compounds were measured with liquid chromatography. A machine learning approach was applied to predict metabolic phenotypes based on community composition, aiming to identify microbial interactions that lead to reductions in ANFs and off-flavour compounds.

### **Results:**

The machine learning models showed varying levels of accuracy across different metabolites, but were effective in identifying trends in microbial metabolic activity. Analysis of model outputs revealed which species -or combinations of species- were associated with distinct metabolic profiles, particularly in the production of volatile compounds. The results demonstrate that the metabolic output of pulse fermentations can be partially predicted based on the composition of the microbial community and that different communities exhibit characteristic metabolic behaviours.

### **Conclusion:**

This work represents a first step toward understanding how phylogenetically distinct micro-organisms interact metabolically during pulse fermentation. By combining fermentation experiments with machine learning, we gained insights into the contributions of individual microbes and microbial combinations to metabolic outcomes. These findings lay the groundwork for the rational design of synthetic communities aimed at improving the sensory and nutritional quality of pulse-based foods.