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Unraveling the gut volatilome dynamics and inter-individual variability during *in-vitro* digestion and fermentation of black beans

Andrea Dell'Olio,[1,2,3] Josep Rubert,[1] Iuliia Khomenko,[2] Martina Moretton,[2] Vincenzo Fogliano,[1] Franco Biasioli [2]

[1] Food Quality and Design, Wageningen University and Research, Wageningen, The Netherlands
[2] Research and Innovation Center, Fondazione Edmund Mach, San Michele all'adige (Italy)
[3] Institute of Materials for Electronics and Magnetism, Consiglio Nazionale delle Ricerche, Povo, 38123 (Italy).

Summary: *This study explores the effect of black beans on the gut volatilome through in-vitro digestion and anaerobic fermentation model systems. Two automated methodologies, HS-SPME-GC-MS and PTR-ToF-MS were employed to analyze VOCs longitudinally. The research highlights the highly dynamic nature of gut fermentation and emphasizes the potential of VOCs as personalized non-invasive biomarkers for real-time metabolic monitoring in gastrointestinal studies.*

Keywords: *gut fermentation, volatilomics, black beans*

Introduction

In Western countries, the increased prevalence of gastrointestinal illnesses poses a significant health burden. To address this issue, a comprehensive understanding of the gut microbiota is required. This knowledge is critical for developing effective methods to manipulate the gut microbiota and reducing the likelihood of metabolic disorders. Volatile organic compounds (VOCs) generated during intestinal fermentation play an important role in host-microbe interactions.[1] There is evidence that frequent consumption of highly fermentable dietary fibers modulates the microflora and gut-associated immune system through the production of gut microbiota metabolites (GMMs) like short-chain fatty acids (SCFAs).[1] Due to the extensive array of molecules produced by the gut microbiota, recent research has focused on identifying new key molecules that have pleiotropic roles. This study investigates the VOCs produced during the fermentation of black beans, a high-fiber, high-protein model food.

Methods

We applied two different automated, non-invasive, techniques: i) solid-phase micro-extraction (SPME) sampling coupled with gas chromatography-mass spectrometry (GC-MS) and ii) proton transfer reaction coupled with time-of-flight mass spectrometry (PTR-ToF-MS) to obtain a continuous monitoring of the gut colonic fermentation.[2,3] Black beans were subjected to static in vitro gastro-intestinal digestion, following the INFOGEST procedure suggested by Brodkorb et al..[4] The undigested pellet was freeze-dried and subjected to batch anaerobic in vitro colonic fermentation.[5] The digested material was fermented by three healthy faecal donors to measure inter-individual variability. Additionally, simulations of ascending and descending colonic conditions were conducted to understand the influence of colonic regions on black beans. Longitudinal multivariate time series analyses, such as empirical Bayes statistics (MEBA) and repeated measures ANOVA Simultaneous Component Analysis (RM-ASCA), were used to identify and select the most relevant VOCs. The complete workflow is presented in Figure 1:
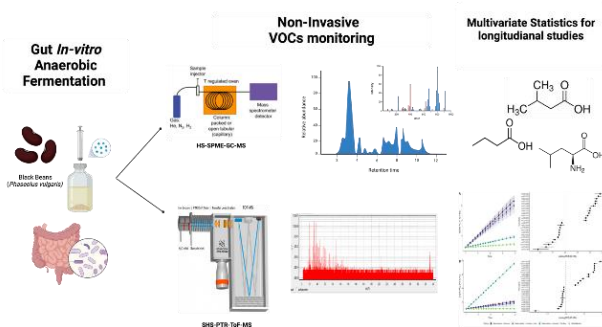

Figure 1. *Non-invasive VOCs monitoring coupled with multivariate statistics for longitudinal assessment of metabolic events taking place during gut microbial batch fermentation of black beans.*

Results and Discussion

By SPME-GC-MS fingerprinting, we detected a total of 156 VOCs. From this screening we identified several compounds exhibiting unique temporal clustering patterns across different donors and colon segments. Our analysis revealed an increased prevalence of sulphur-containing compounds specifically in samples obtained from the descending compartment of the colon. Additionally, by employing PTR-ToF-MS, distinct quantitative differences when comparing VOCs across individual donors were identified. These differences highlight the variability in microbial metabolic responses which is dependent on the type of dietary substrate but also the inherent microbial communities of faecal donors. The same substrate can lead to diverse metabolic profiles, potentially resulting in varied impacts on the host's health and metabolism. Overall, our findings underscore the significance of VOCs as personalized, non-invasive biomarkers for real-time metabolic monitoring in gastrointestinal research. The ability to longitudinally monitor these compounds provides valuable insights into individual metabolic responses and could serve in developing tailored dietary interventions and therapeutic strategies. This personalized approach could enhance our understanding of host-microbiome interactions and their implications for health and disease.

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