NOTA: Saranno inclusi nel libro degli atti **esclusivamente** gli abstract conformi al template

ABSTRACT SUBMISSION FORM

To be sent *to*

segreteria@torino2023.spettrometriadimassa.it

Presenting Author

Surname and name: Dell'Olio Andrea		
Company or Organization: *Wageningen University and Research (NL), Edmund Mach Foundation (IT), National Research council (IT)*		
Address *Bornse Weilanden 9, 6708 WG, Wageningen,The Netherlands* *Via Edmund Mach, 1, 38098 TN, San Michele All'adige, Italy*		*Zip code – Town – Country* *6708 WG Wageningen The Netherlands* *38098 San Michele All'adige, Italy*
Telephone *+393455143405*	*Fax*	*E-mail* *andrea.dellolio@wur.nl*

Preference: ☒ ***Oral presentation*** ☐ ***Poster***

Title

On-line tracking of the human gut microbial metabolism:
high-throughput screening during colonic *in-vitro* fermentation

Authors

[1,2,3] Dell'Olio Andrea, [1]Rubert Josep, [1]Fogliano Vincenzo, [2]Khomenko Iuliia, [2]Betta Emanuela, [3]Capozzi Vittorio, [2]Biasioli Franco

Company(es) or Organization(s)

[1] Food Quality and Design, Wageningen University and Research, The Netherlands
[2] Research and Innovation Center, Fondazione Edmund Mach, Italy,
[3] National Research Council, Italy

On-line tracking of the human gut microbial metabolism: high-throughput screening during colonic *in-vitro* fermentation

[1,2,3] Dell'Olio Andrea, [1]Rubert Josep, [1]Fogliano Vincenzo, [2]Khomenko Iuliia, [2]Betta Emanuela, [3]Capozzi Vittorio, [2]Biasioli Franco

[1] Food Quality and Design, Wageningen University and Research, The Netherlands
[2] Research and Innovation Center, Fondazione Edmund Mach, Italy,
[3] National Research Council, Italy

Summary: The human gut encloses a large community of bacteria producing a wide range of volatile organic compounds (VOCs) when fermenting undigestible substrates. This study aims to provide a high throughput method to study in real-time the gut microbial volatilome when the microbiota process undigestible dietary substrates.

Keywords: *Gut Microbiota, online VOCs monitoring, HS-SPME-GC-MS, SHS-PTR-ToF-MS*

Background: Small metabolites from the human gut microbiota are recognized as the intermediates of the microbiome-host cross-talk [1]. The research on the human gut metabolome is mainly based on discrete sampling representing discontinuous 'snapshot' of these complex biological systems [2]. The aim of this research work is to enhance the current understanding of the dynamics of the gut microbiota by integrating non-invasive and continuous analytical methods with *in-vitro* gut simulators, to monitor in real-time, the progression of small molecules released into the headspace [2,3]

Methodology: Automated Head space-Solid Phase Micro Extraction coupled with Gas Chromatography-Mass Spectrometry (HS-SPME-GC-MS) and Static Headspace- Proton Transfer Reaction-Time of Flight-Mass Spectrometry (SHS-PTR-ToF-MS) are used for the purpose of this investigation. The objective is to screen and monitor a specific set of masses of interest, to gain system level mechanistic insights on primary metabolism of the gut microbial consortia.

Results: This methodology enabled the continuous monitoring of multiple metabolites in time, including short-chain fatty acids (SCFAs) and medium-chain fatty acids (MCFAs) derived from 24h oat bran fermentation. A mixture of -odd and -even chain acids were co-released into the culture headspace after 4 hours of fermentation and their relative abundance increased in time over 24 hours. The production of multiple MCFAs from the substrate is most likely a community optimization strategy to maximize ATP production from oat degradation by means of reverse beta-oxidation which involves the utilization of fermentation intermediates, such as propanol and acetate. Furthermore, the untargeted screening allowed the detection of low abundant sulfur metabolites, thiophenes, which, to our knowledge, were never investigated before as gut microbial metabolites (GMMs).

Conclusion: By integrating non-invasive and continuous analytical methods with an *in-vitro* gut simulator, it was possible to monitor in real-time the progression of two important class of small molecules released by the microbial consortia into the headspace. The collected information can be jointly integrated to shed light on the dynamics of bacterial foraging of complex undigestible substrates (e.g. bran from cereals). Overall, these results confirm the idea to consider the bacterial headspace as a highly dynamic chemical system that contains information on microbial community behavior.

References:

1. Donia, M. S. and Fischbach, M. A. (2015). Small molecules from the human microbiota. Science 349,1254766
2. Meredith, L. K., & Tfaily, M. M. (2022). Capturing the microbial volatilome: an often overlooked 'ome'. Trends in Microbiology, 30(7), 622-631.
3. Biasioli, F., Gasperi, F., Yeretzian, C., & Märk, T. D. (2011). PTR-MS monitoring of VOCs and BVOCs in food science and technology. TrAC Trends in Analytical Chemistry, 30(7), 968-977.
4. Pérez-Burillo, S., Molino, S., Navajas-Porras, B., Valverde-Moya, Á. J., Hinojosa-Nogueira, D., López-Maldonado, A., ... & Rufián-Henares, J. Á. (2021). An in vitro batch fermentation protocol for studying the contribution of food to gut microbiota composition and functionality. Nature protocols, 16(7), 3186-320