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# Real-Time Volatile Profiling by Direct-Injection Mass Spectrometry: A Sustainable Strategy to Accelerate R&D in Fermentation Studies

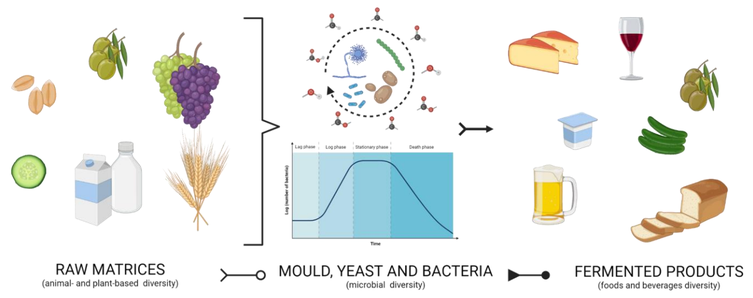
Mariagiovanna Fragasso[1*], Vittorio Capozzi[2], Giuseppe Spano[1], Franco Biasioli[3]

[1]Department of Agriculture Food Natural Science Engineering, University of Foggia, Via Napoli 25, 71122, Foggia, Italy

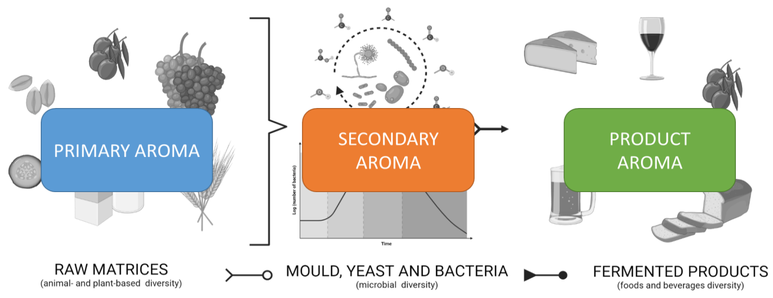
[2]Institute of Sciences of Food Production, National Research Council (CNR), c/o CS-DAT, Via Michele Protano, Foggia, Italy

[3]Fondazione Edmund Mach, San Michele all'Adige (TN), Italy

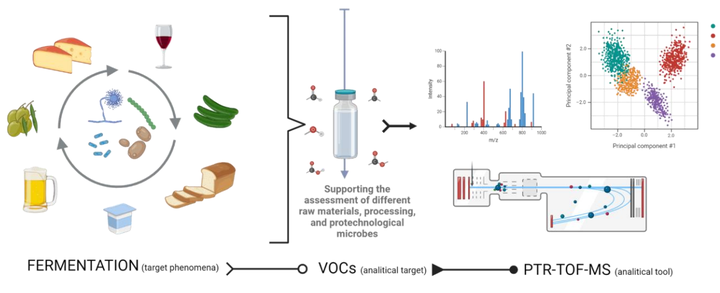
*Corresponding author: mariagiovanna.fragasso@unifg.it



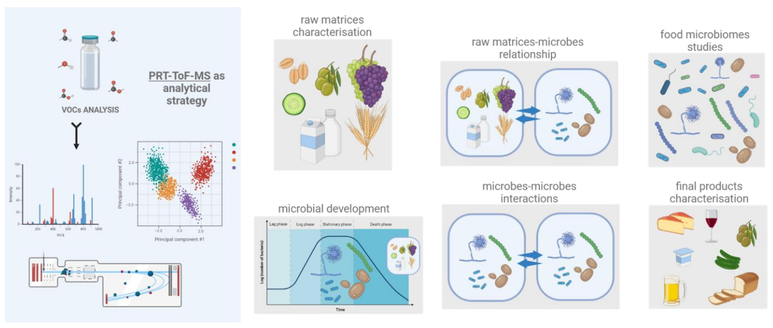

The term 'fermentation' summarises a family of bioprocesses of huge interest for the green transition of food systems. Among the changes induced by microorganisms, there are improvements in sensory quality. In this light, the flavour is one of the principal targets of the transformative power of fermentation.



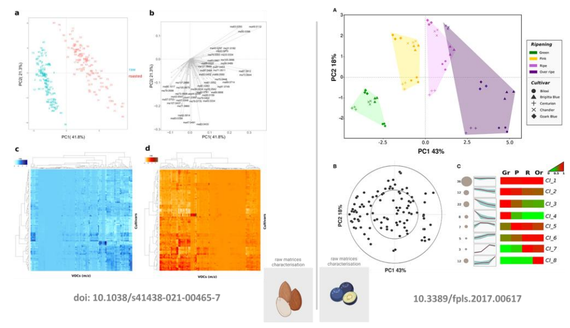

Among the compounds responsible of flavouring properties, some are associated with the raw materials, while others with the fermentative process. Hence, volatile organic compounds provide chemical information about the quality of the fermented products and concerning the progress of microbial-based bioprocesses in food.



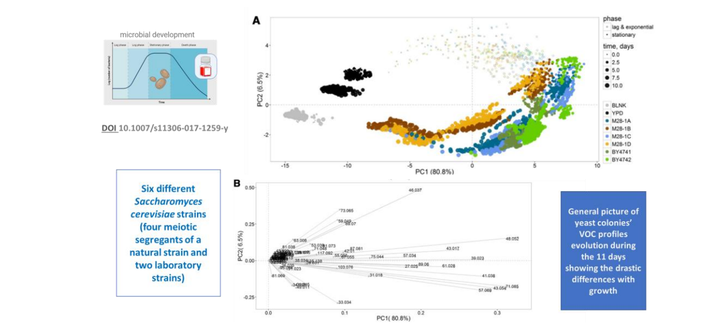

VOCs represent versatile targets for an integrated assessment and PTR as an analytical strategy presents strengths that are well connected to the need to carry out monitoring over time and massive screening to support research and development in the case of bioprocesses that see a high number of variables involved.




We have collected the different variables into 6 macro-categories: (i) raw matrices characterization, (ii) microbial development, (iii) raw matrices-microbes relationship, (iv) microbial interactions, (v) food microbiomes studies, (vi) final products characterization. You will find references to these categories in the next slides/case studies.




The possibilities of rapid screening of different cultivars, maturity levels, and processing aptitudes are interesting for the design of new products and the optimization of fermented foods and beverages. New avenues are opening up for the economic valorization of biodiversity through fermentation, with application relevant in the food industry.




Yeasts are fundamental organisms for the flavor and quality of fermented products. The analytical strategy is useful to perform a monitoring that without a rapid and non-destructive approach, would require the setup of a complex experimental test and very expensive analyses. *S. cerevisiae* is also a model organism in biology studies.

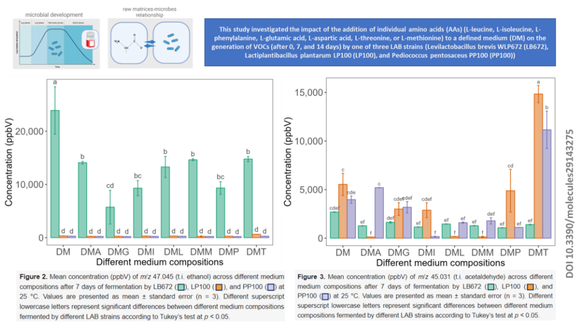
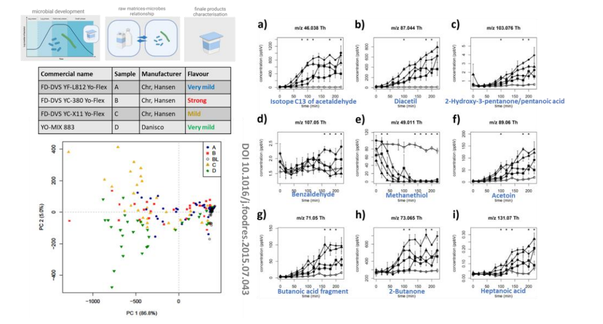


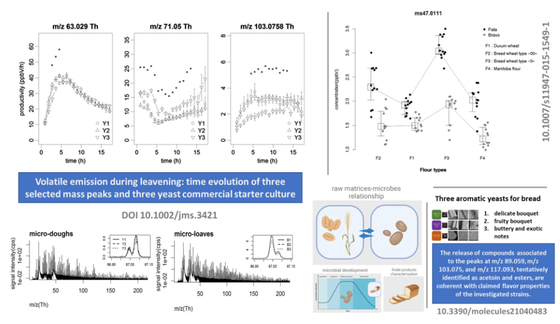

**Figure 2.** Mean concentration (ppbV) of *m/z* 47.045 (ℓ.ℓ ethanol) across different medium compositions after 7 days of fermentation by LB672 (■), LP100 (■), and PP100 (■) at 25 °C. Values are presented as mean ± standard error (n = 3). Different superscript lowercase letters represent significant differences between different medium compositions fermented by different LAB strains according to Tukey's test at $p < 0.05$.

**Figure 3.** Mean concentration (ppbV) of *m/z* 45.031 (ℓ.ℓ acetaldehyde) across different medium compositions after 7 days of fermentation by LB672 (■), LP100 (■), and PP100 (■) at 25 °C. Values are presented as mean ± standard error (n = 3). Different superscript lowercase letters represent significant differences between different medium compositions fermented by different LAB strains according to Tukey's test at $p < 0.05$.

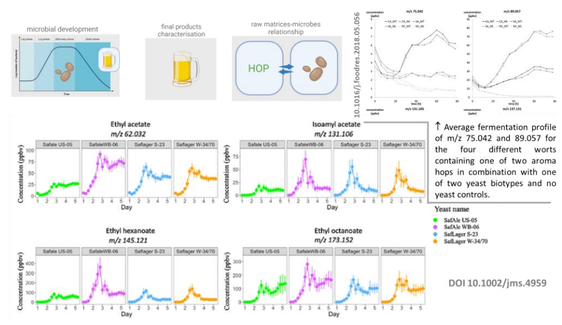
The possibility of having a low-cost and non-time-consuming system allows for more deterministic evaluations of volatile formation pathways, using simple chemically defined media integrating the potential substrates individually. These evaluations have shown some species-specific trends demonstrating the need for tailored studies to improve flavour.



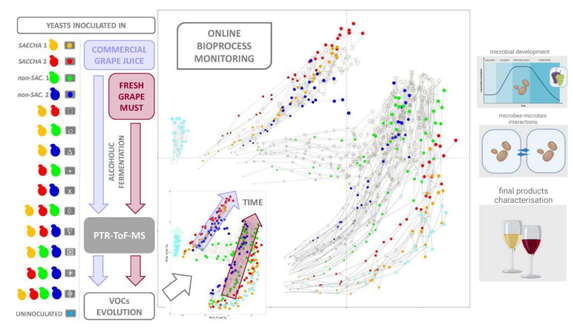

The starter culture industry for fermented foods is of global importance. Flavoring attributes are particularly interesting. We have explored starter cultures for yoghurt defining a panel of markers and trends useful for future screening. The untargeted potential of the technique has allowed us to define a new potential positive trait (reduction of methanethiol).



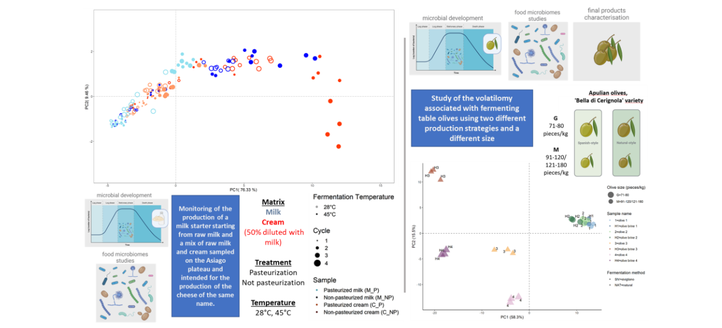

Similarly we did for yeasts. In addition to monitoring and defining markers, we also explored the effects of diverse combinations of starter cultures and flours on the different peaks. PTR also proved useful in elucidating the molecular basis of particular aromatic yeasts capable of imparting fruity aromas, exotic notes and butter to bread.



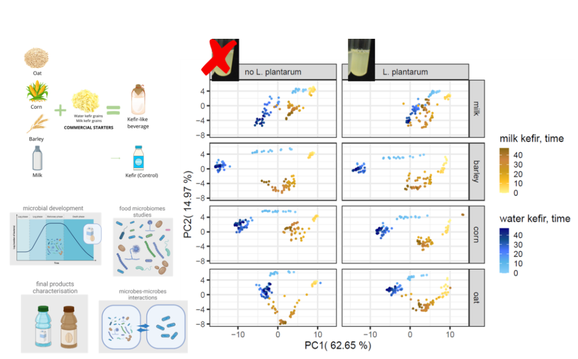

Also for the brewing industry, rapid monitoring of different markers useful to understand and optimize the contribution of yeasts. Evaluation of the influence of different hop varieties, to support the yeast/flour evidence in bread production. Thanks to the versatility of the technique and the low cost, it is possible to offer R&D paths also for the craft beer sector.



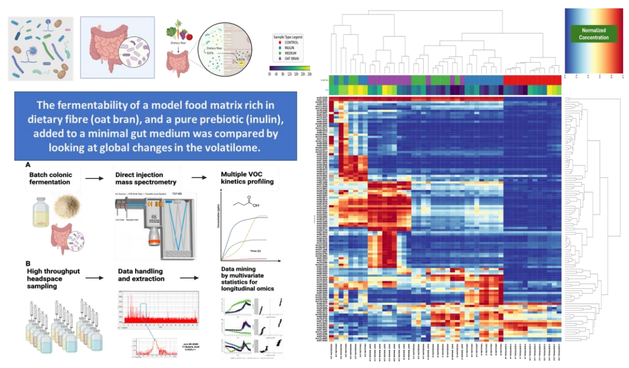

In the starter culture industry, particularly in winemaking, the combination of bio-resources to meet specific needs of the sector is increasing. PTR has proven useful in studying different combinations of *Saccharomyces* and non-*Saccharomyces*, providing information on sensory diversification and dominance phenomena in time.



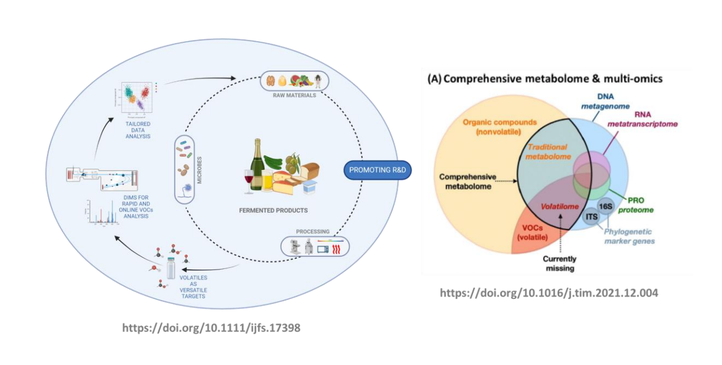

For several traditional products, fermentations are based on the development of autochthonous consortia, which represent so-called microbiomes. Growing interest in microbiomes is orienting towards the study of communities of microorganisms. Very interesting that DIMS allow an 'omics' in its temporal evolution.




this is also the case for kefir. The evolution of microbiomes in different matrices, also through cross-over solutions, for the design of plant-based beverages has been the subject of study. The evaluation of the disruptive potential of a single strain used for biofortification action can also be deduced from the trends highlighted.




Microbial-based modifications do not stop at food fermentations. Even in our intestines bio-conversions occur that can be monitored with PTR, taking advantage of the potential of the technique, offering an untargeted overview that does not stop at short-chain fatty acids. It is interesting to evaluate the fate of microbiomes, probiotics and prebiotics.

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An analytical strategy to optimize the flavour in fermented products and to use VOCs as a driver for sustainable improvement of quality and safety of fermented products. For multi-omics approaches, PTR volatilomics can provide information on the temporal evolution of phenomena, realizing opportunities for foodomics and microbiome studies.

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