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Monitoring the volatilome of kefir and kefir-like cereal-based beverages during fermentation by PTR-Tof-MS

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Summary: *Enhancing fermentative bioprocesses is a needed step towards higher environmental, social, and economic sustainability standards. Functional beverages, milk kefir in particular, stand out as a rapidly expanding segment for their health benefits. This study demonstrates the feasibility of green real-time monitoring of volatile profile kefir-like beverages during fermentation.*

Keywords: *omics, lactic acid bacteria, functional food*

Introduction

Functional beverages stand out as a rapidly expanding segment within the realm of emerging food categories. Milk kefir, an ancient traditional fermented beverage, holds a place within the domain of functional foods, acknowledged for its established edonistic and health-enhancing properties. Milk kefir is commonly produced using a combination of lactic acid and alcoholic fermentation by microbial flora, conferring a small amount of ethanol content and a unique characteristic flavour to this product. The traditional method of making milk kefir draws in kefir grains that are mixed cultures consisting of various lactic acid bacteria yeasts, and acetic acid bacteria that co-exist in a symbiotic association and are responsible for an acid alcoholic fermentation [1,2].
Different formulations and biotechnological innovations were recently proposed to develop kefir and kefir-like products with improved sensory, nutritional, and functional features [3]. For instance, kefir grains were used to ferment non-dairy raw materials, such as vegetables and cereals to produce functional beverages enriched in vitamins, bioactive compounds or able to vehicle potential health-promoting bacteria.
In this contest, volatile organic compounds (VOCs) serve as valuable indicators to monitor bioprocesses, offer insight into the quality of the matrices and can be considered as promising biomarkers in terms of sensory properties. Proton-transfer-reaction, coupled with Time-of-Flight Mass Spectrometer (PTR-ToF-MS), represents a green, rapid and non-invasive analytical solution to screen microbial volatilome and was widely applied in the field of fermentation monitoring [4].
This work aimed to evaluate the dynamic changes of volatile profile of products obtained during the fermentation by PTR-ToF-MS coupled with a multipurpose GC automatic sampler.
In the present research different matrices were selected as two case studies: raw milk and UHT milk (case study 1) and oat, maize and barley flours (case study 2).

Experimental

Case study 1: raw milk and UHT milk were fermented with a commercial starter (milk kefir preparation). VOCs produced during fermentation were sampled automatically every 3 hours for 48 hours at 26°C by static headspace module of multipurpose GC automatic sampler (Gerstel GmbH, Mulheim am Ruhr, Germany) into a PTR-ToF-MS 8000 device (Ionicon Analytik GmbH, Innsbruck, Austria).
Case study 2: standard milk–based kefir (as control) and three cereal-based kefir-like beverages, obtained from oat, maize and barley flours, were fermented with commercial starters: water kefir preparation and milk kefir preparation with or without the addition of *Lactoplantibacillus plantarum* strain, according to the method previously reported by Yépez et al. [3]. VOCs produced during fermentation were sampled automatically every 2 hours for 48 hours at 26°C by a dynamic headspace module of multipurpose GC automatic sampler into a PTR-ToF-MS 8000 device.
All PTR-ToF-MS data collected were processed and analyzed using the procedure described by Cappellin et al. [5].

Results

Changes of variability in the time of the inoculated samples were observed in more than 300 mass peaks at significant concentrations. The differences in the matrix formulation, starter and additional inoculation within the kefir beverage types under investigation affected their VOCs profiling in all samples. The formation of

volatile higher alcohols and corresponding esters during kefir fermentation was influenced by the composition of the starter and inoculation with Lactoplantibacillus plantarum. Considering different matrices, the inoculation has a higher influence on the VOCs profiling of corn and oat kefir than on of barley and milk beverages. The evidence helps to underline the role of volatiles in the study of the development of protechnological and spoilage microbes (raw milk trial). The dominance of the starter over the evolution of the psychrotrophic microflora has also been assessed.

Conclusions

The evidence gathered in the present study indicated that PTR-ToF-MS measurements effectively assess and monitor VOCs during fermentation processes, allowing for the tracking of their evolution and kinetics. By detecting significant VOCs produced by microbial metabolism, such as higher alcohols, esters, volatile fatty acids, and sulfur compounds, our ability to customize food flavor is enhanced. This includes gaining a better understanding of flavor-active molecules and developing new methods for reducing off-flavors. Simultaneously, VOC monitoring can aid in leveraging other pro-technological and beneficial aspects of microbial physiology, thereby improving the functionality and safety of the product. Additionally, the observed differences in VOC profiles among kefir beverage types are expected to result in varying sensory properties, particularly influencing consumer overall liking. The potential of the technique for monitoring undesired microbial growth was also explored, highlighting possible uses for safety monitoring, limiting spoilage phenomena, and for rapid information on microbial starter dominance.

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