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The "form" makes the difference: comprehensive two-dimensional gas chromatography as highly informative fingerprinting tool to study the impact of fructose formulation on mice's microbiota and its volatile metabolome signatures

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Introduction:

Fructose has been the focus of several studies for its potential adverse health effects. Although fructose is a monosaccharide naturally present in many fruits, most of the dietary intake derives from "hidden" sources [1-2]. Fructose intake alters microbiota composition, resulting in reduced bacterial diversity and altered expression of genes involved in specific metabolic pathways [3]. The fecal volatile metabolome is an expression of gut microbiota composition and, in an indirect way, of the available substrates reaching the gut; its chemical composition, i.e. fingerprint, is thereby suggestive of metabolic changes induced and/or caused by microbial activity. Comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC-TOF MS) in combination with advanced fingerprinting based on pattern recognition, represent the most informative analytical platform to investigate complex fractions of volatiles in great detail.

Methods:

GC×GC-TOF-MS was performed on a system equipped with a loop-type thermal modulator (Zoex LCC, USA) combined with a fast time-of-flight mass spectrometer featuring tandem electron impact ionization at 70 and 12 eV (Markes Select-eV, UK). Combined Untargeted and Targeted fingerprinting was by pattern recognition based on template matching (GC Image LCC, USA).Four-week-old male C57BL/6J mice (n=18) were divided into three diet groups: a group fed by standard diet and drinking tap water (CS group, n = 6), a group fed a standard diet and drinking a 60% fructose syrup (FL group, n = 6), and group fed a 60% fructose solid diet and drinking tap water (FS group, n = 6), for twelve weeks. After 12 weeks of diet intervention, small intestinal, fecal and colonic content were collected, immediately frozen in liquid N_2 and stored at -80°C for volatiles fingerprinting and metagenomic analysis.

Results:

The volatiles sampled by headspace solid phase microextraction (HS-SPME) from fecal samples collected by mice undergone to dietary intervention, were submitted to informative fingerprinting by GC×GC-TOF-MS. 2D patterns were characterized by, on average, 400 detectable analytes including several classes of chemical such as: hydrocarbons, alcohols (short-chain and long-chain alcohols derived from acids reduction), carbonyl derivatives (aldehydes and ketones), short-chain fatty acids (acetic, propionic, butyric above all), branched-chain fatty acids, aromatic compounds (including heterocycles) and monoterpenoids. The relative distribution of volatiles between samples proved to be informative of differential diet intervention. Clustering was clear between diet regimens (CS vs. fructose-enriched diets – FS and FL) and several compound classes resulted up- or down-regulated in function of the type of sugars (starch vs. fructose) and its administration form (liquid vs. solid).

Above all, butyric and propanoic acid esters (butyl butanoate, 2-methylbutyl propanoate, 1-butanol-3-methyl propanoate) were up-regulated in fructose-enriched diets, while free SCFAs (acetic, propanoic and butanoic acids) were more abundant in feces from standard diet mice.

Low electron ionization, limiting analytes fragmentation, enabled to achieve higher Signal-to-Noise ratio values for several analytes providing information complementary to the hard ionization traces (i.e., 70 eV). Thanks to the characteristic rational elution pattern of 2-methyl ketones, the series

of degradation products of *iso*- and *anteiso*- fatty acids was clearly delineated providing additional information about bacterial metabolism in the gut [4].

Conclusions:

Most of the analytes revealed to be informative of the differential impact of diet were confirmed by literature data while a group of degradation products of branched fatty acids was for the first time detected in such samples. Chemical signatures were also coherent with metagenomics data on microbial populations. The investigation approach based on advanced fingerprinting by GC×GC-ToF-MS and pattern recognition confirms its suitability to exploit samples with multiple classes of chemicals providing data to better understand the complex biological phenomena triggered by diet composition and microbiota.

References

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