Coupling solid phase microextraction to complementary separation platforms for metabolomics of E. coli metabolome in response to natural antibacterial agents

Fatemeh Mousavi, Emanuela Gionfriddo, Eduardo Carasek, Erica A. Souza-Silva, Janusz Pawliszyn
Department of Chemistry, University of Waterloo, Ontario, Canada, N2L 3G1

Introduction
Owing to their antimicrobial characteristics, essential oils are widely used in medicine as well as in the food and fragrance industries. Among the essential oils constituents, oxygenated terpenoids such as alcohols and phenolic terpenes have demonstrated the highest antimicrobial activity potential. Moreover, oils derived from bay, cinnamon, clove, and thyme yielded the highest bactericidal effect when applied as antibacterial agents against five food-borne pathogens (Campylobacter jejuni, Salmonella enteridis, E. coli, Staphylococcus aureus, and Listeria monocytogenes). The interactions between different constituents of essential oils may lead to synergistic, antagonistic, indifference, or additive effects. The mechanisms of action of these naturally occurring compounds against pathogens are still not fully understood, especially at the molecular level. As the metabolome of living systems is a response to environmental stress, metabolomics platforms aim to provide comprehensive information to genomics, transcriptomics, and proteomics. Within this context, global analysis of all metabolites in a given system can be employed to discover potential biomarkers of effects or reactions attributable to specific stimuli. Due to the broad chemical and physical characteristics of metabolites, no single analytical platform could provide identification of all metabolites present in a living system. In addition, to increase method sensitivity and provide wider metabolome coverage, a proper sample preparation strategy, able to obtain the most representative, yet clean extract possible, needs to be used. In this context, solid phase microextraction (SPME), as one of the recently emerging techniques in sample preparation for metabolomics studies, is capable of fulfilling many of the criteria for ideal sample preparation in metabolomic investigations such as non-selectivity, reproducibility, simplicity and possibility for automation.

The purpose of this study was to employ SPME coupled to GCxGC-ToF/MS and UPLC-HRMS and bioinformatics tools to study changes in metabolic pathways of E. coli metabolome submitted to treatment with clove bud oil and its major constituents. Moreover, multivariate experimental design was applied to optimize extraction and sample preparation factors that impact extracting and to evaluate the type of interactions occurring between the major active components of clove oil. Individual components of the clove oil were characterized and identified. Finally, the metabolic profiles of antibacterial agent-treated cells and control cells were generated by both optimized platforms and subjected to multivariate data analysis. These metabolic patterns produced clear separation between controls and treated samples on an orthogonal projections to latent structures discriminant analysis (OPLS-DA) analysis due to up-regulated and down-regulated metabolites.

Sampling devices and instrumentation
GC analysis: for HS-SPME of E.Coli cultures a DVB/CAR/PDMS fiber coating was used for extraction and a GCxGC-ToF/MS Pegasus 4D (LECO Corporation) for separation and detection of the analytes. First and second dimension GC capillary columns were Rtx-85SISMS (30 m x 0.25 mm x 0.25µm) (Restek Corp., Bellefonte, PA, USA) and BP-20 (1 m x 0.1 mm x 0.1 µm) (SGE, Austin, TX, USA), respectively.
LC analysis: extractions were performed using a 96-blade SPME system consisted of PDS-DVB-WAX/XLB 50:50 [w/w] as extraction phase coated onto stainless steel blades. For analysis, an UPLC-Exactive (Thermo Fisher Scientific) equipped with a Kinetex pentasilphenylporphyrin coreshell column (1.7 µm, 2.1 mm x 10 mm) (Phenomenex) was used.

Clove oil components antibacterial efficiency

Metabolic profiling by SPME and UPLC-HRMS
Metabolic profiling of E. Coli under different compositions of antibacterial agents using 96-blade SPME system coupled to UPLC-HRMS.

10,000 features were detected in both EB+ and EB- modes. Almost 60 % of the peaks showed statistically significant changes due to treatment with antibacterial agents.

The hydroxy group present in the molecule plays an effective role in the prevention of bacterial growth.

Effect of clove oil and eugenol on VOC profile of E. Coli

Metabolic profiling of E. Coli under different compositions of antibacterial agents using HS-SPME-GCxGC-ToF/MS

Score plot corresponding to OPLS-DA analysis of a media-bacteria (green dots) versus media-bacteria-clove (red dots) and media-bacteria (green dots) versus media-bacteria-eugenol (blue dots) and corresponding P-values (c) and (d)

- the compounds most affected by treatment with antibacterial agents were derivatives formed along the fatty acid biosynthetic pathway
- decrease in indole, triazole, methanethiol, and butanone levels demonstrate disruptions in tryptophan, thiamine, cysteine, methionine (and other amino acid containing sulfur), and riboflavin metabolism, respectively. These alterations in the volatilomic profile could be attributed to the inhibition effect of eugenol on enzymes such as tryptophanase and lipoxigenase, due to deamination and termination or reduction of decarboxylation
- the aldheydes whose concentrations were mostly affected by treatment with clove oil and eugenol were furfural, benzaldehyde, and nonanal. Benzaldehyde production can be related to two different pathways, leading to the conversion of L-phenylalanine to benzoyl-CoA, whereas nonanal arises from metilketones

In the respiratory pathway of E. coli could be monitored by investigating metabolites with a high vapor pressure escaping from liquid media with the use of headspace SPME

Conclusions

- Suitability of Solid-Phase Microextraction as a reliable tool to capture variations of E. coli metabolome and volatilome in response to natural antibacterial agents such as clove oil and its major component eugenol was demonstrated
- The use of SPME coupled to GCxGC-ToF/MS and UPLC-MS provided a comprehensive metabolome snapshot of metabolites with a wide variety of physical and chemical characteristics, including volatiles, polar, and nonpolar metabolites, thus enhancing the amount of chemical information retrievable from the system under investigation in comparison to conventional extraction techniques.

Acknowledgments
- Dr. Richard Smith, Nathaly Reyes-Garces, Dr. Barbara Bojko, Dr. Angel Rodriguez Laffuente, and Dr. Seliena De Grazi
- Olivier Niquette and KC Walkbin for the technical support with the GCxGC-ToF/MS
- To our sponsors:

References