

## CO.3 Bioanalytical application of headspace-SPME: the investigation of *Candida albicans* and *Candida dubliniensis* signalling molecules

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During the last years biological sciences are increasingly benefitting from the contribution of chemical principles and tools. In fact, methodologies primarily developed for the chemical field saw their application scope widely enlarged to biology, biochemistry and medicine – bioanalytical chemistry. An example is Headspace-Solid Phase Micro Extraction (HS-SPME) that offers many advantages, including small sample volume, simplicity, speed and solventless extraction.

The present work was based on a biological problem that was solved using a tool traditionally applied in the chemical field. Specifically, from a biological point of view, this study focused on morphogenic compounds produced *in situ* by *Candida albicans* and *Candida dubliniensis* during planktonic and biofilm growth that may at least partially substantiate the effect promoted by culture supernatants in those yeasts morphogenesis. In order to get insights into supernatants composition, it was necessary to identify and quantify their components, specifically the alcohols. For both species, planktonic vs. biofilm supernatant alcohols were extracted using HS-SPME and analysed by gas chromatography-mass spectrometry (GC-MS). HS-SPME-GC-MS methodology previously developed to analyse the volatile composition of fruits was followed<sup>1</sup>. The identification of *Candida* metabolites was achieved comparing the GC retention times and mass spectra, with those of the pure standard compounds. All mass spectra were also compared with the data system library (Wiley 275). A comparable analysis was done with growth medium and no interfering substances were found into or near the retention times of those compounds. The quantification was performed by preparation of growth medium solutions containing pure standards in the same conditions of the samples. For each compound, appropriate concentration ranges were chosen in order to include sample concentrations. Standard curves were generated for GC-MS peak areas *versus* concentration of each compound ( $R^2 > 0,98$ ), with quantification relying above quantification limits.

Both planktonic cells and biofilm supernatants of *C. albicans* and *C. dubliniensis* contained isoamyl alcohol, 2-phenylethanol, 1-dodecanol, *E*-nerolidol and *E,E*-farnesol, obtained at good chromatographic resolution conditions. Alcohols secretion profiles were species, culture mode and growth time specific. The biological activity of these compounds on *C. albicans* and *C. dubliniensis* morphogenesis was proved, showing the role of those alcohols in morphogenesis signalling.

This work evidences that the chemistry perspective of molecules is fundamental to explain complex behaviours in biological systems.

- 1 Coelho, E.; Rocha, S.M.; Delgadillo, I.; Coimbra, M.A. Headspace-SPME applied to varietal volatile components evolution during *Vitis vinifera* L. cv. 'Baga' ripening, *Anal. Chim. Acta*, 2006, 563, 204-214.