# Easy and Accurate Methodology to Quantify Volatile Compounds in Fermented Beverages 

Oliveira J.M..${ }^{1}$, Genisheva Z. ${ }^{1}$, Lima L. ${ }^{1}$, Vilanova M. ${ }^{2}$<br>${ }^{1}$ IBB-Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, Universidade do Minho, 4710-057 Braga, Portugal<br>${ }^{2}$ Misión Biológica de Galicia (CSIC), PO BOX 28, 36080 Pontevedra, Spain<br>*Corresponding author: jmoliveira@deb.uminho.pt

Volatile compounds of fermented beverages may derive from raw matter, but the majority are formed during fermentative processes. In the some specific cases, e. $g$. wines, they may also arise during conservation and ageing. These volatiles belong to a diversity of chemical families, including alcohols, esters, fatty acids, volatile phenols, terpenes and norisoprenoids. Levels of individual compounds may range from few $\mathrm{ng} / \mathrm{L}$ to hundreds of $\mathrm{mg} / \mathrm{L}$. Although major volatiles may be analysed directly by gas chromatography, most of them needs a previous step of concentration. Concentration was traditionally achieved by solvent extraction, liquid-liquid mixture, soxhlet and SPE, followed by evaporation; more recently solvent-free extractive techniques SPME and SBSE, became usual due to their detection limits and selectivity. However, these techniques have some drawbacks: environmental aspects when using large quantities of solvents, price, needs of specialized equipment and workmanship. Furthermore, chromatographic detectors may be selective, e. g. pulse flame photometric detector (PFPD) for sulphurous compounds or nitrogenous compounds, or somewhat universal as mass spectrometer (MS). However, although MS permits the quantification of a wide range of volatiles, the equipment is very expensive and cost-maintenance. Another detector largely used by research centres and institutions linked to beverages sector, the flame ionisation detector, acquire attractiveness due to its good response to the methyl groups of organic compounds and its appellative price.
These constraints makes difficult to achieve a universal methodology to analyse, in a single and quick approach, all the constituents of a fermented beverage like wine, beer or other products obtained by the fermentation of fruit juices. Moreover, when analysts of research centres and tutelary institutions need to compare samples, to quantify some few key volatile compounds or even to confirm its authenticity, they aspire for an easy and accurate methodology, which additionally uses inexpensive equipment.
This work refers to the implementation of a quick methodology to quantify volatile compounds of fermented beverages, carried out by liquid-liquid micro-extraction followed by chromatographic analysis. The optimized methodology, reached to maximize the number of extractions per day, only uses 8 mL of sample and $400 \mu \mathrm{~L}$ of dichloromethane. Each battery of extracts, ready for chromatographic analysis by GC-FID, may be obtained after 30 min , and only uses ordinary labware.
Although the proposed methodology may permit the identification and quantification of a large number of compounds, it was implemented for 40, belonging to the following families: alcohols, esters, terpenes, volatile fatty acids, volatile phenols and norisoprenoids. Validation was performed in terms of linearity, limits of detection and quantification, repeatability, reproducibility and recovery.

