

## Introduction

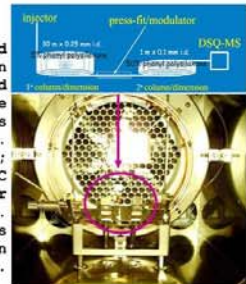
Gas chromatography (GC) is the most powerful technique used for the separation and determination of (semi)volatile compounds. However, the complexity of the aroma profile of a food product often results in chromatographic traces of difficult interpretation (multitude of trace components, overlapping peaks). Comprehensive two-dimensional gas chromatography (GCxGC) offers enhanced separation and identification performance of the aroma components in a single run, due to higher peak capacity, two different separation principles (one for each chromatographic dimension) and an improved signal-to-noise ratio compared to 1D-GC. Thus, GCxGC is a promising technique for new applications in aroma research and for determining beer authenticity. In a GCxGC trace, chemically correlated compounds are reported as homogeneous groups in a 2-dimensions plot. This allows to obtain specific separation patterns which simplify the identification of volatiles. In addition, GCxGC can be coupled to Dynamic Headspace Solid Phase Microextraction (DH-SPME) for beer quality control and authentication. The application of SPME-GCxGC-MS for the characterization of the aroma profile of Spelt, Pils and double bock beers was discussed.

## Materials and methods for data collection

A divinylbenzene/carboxen/polymethylsiloxane (DVB/CAR/PMDS) fiber, 2 cm length, was chosen. Five millilitres of each beer were poured in a 15 mL vial together with 1 g NaCl and 50 µL of the standard solution (4-methyl-2-pentanol, 1 g/L) and equilibrated for 10 min at 40°C. The SPME device was manually inserted into the sealed vial containing the sample and the fiber was exposed to the headspace for 30 min at 40°C. A nitrogen flow, into the vial, was used to increase the transfer of the volatiles to the fiber. After sampling, the SPME device was immediately introduced into the injection port of a GCxGC, coupled with a qMS.

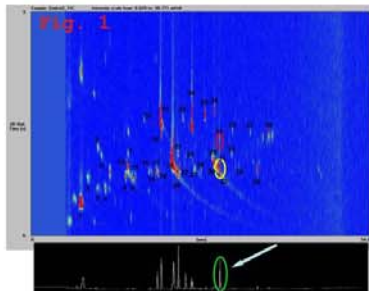


The thermal desorption lasted for 5 min at 230°C. The 1st column (30m x 0.25mm x 0.25µm) was coated with 5% phenyl polysiloxane and the 2nd column (1m x 0.1mm x 0.1µm) was coated with 50% phenyl polysiloxane. The GC program was: 50°C (1 min); to 210°C at 4°C/min, then to 250°C at 5°C/min and finally held for 5 min. Helium was used at 0.8 mL/min. The modulation period was 3 s and the DSQ operated at a scan rate of 16 scan/s at 40-300 m/z.

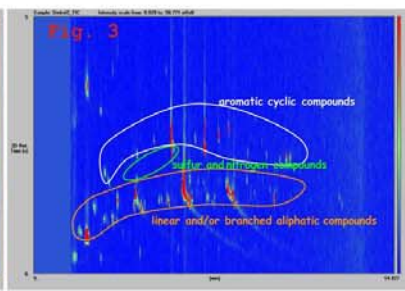
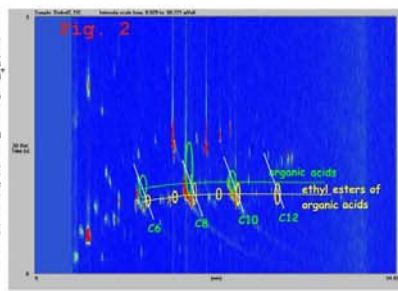


## Results

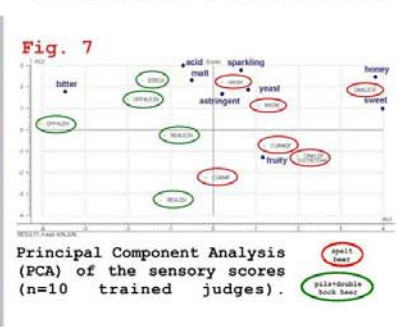
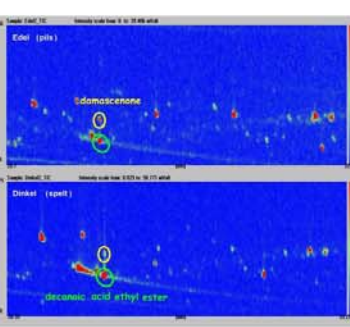
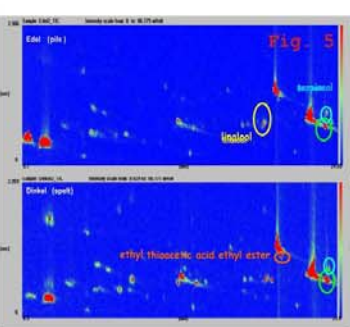
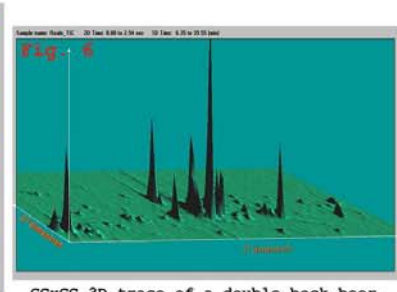
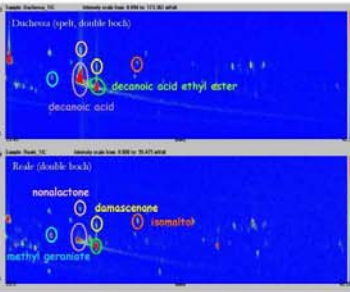
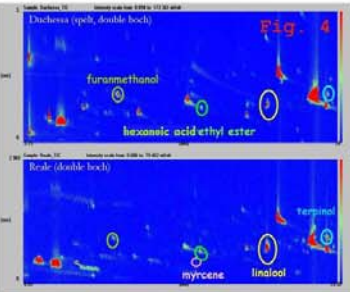
The 2D-GC trace (Fig.1) shows a clear distribution of the volatiles due to the non-polar/medium polar phase combination. A great number of volatile compounds, belonging to very heterogeneous groups such as alcohols, esters, organic acids, terpenes, sulfur compounds, phenols have been identified in all beers; each beer type showed a typical composition. Fig. 1 shows the peak capacity of the GCxGC system compared to 1D-GC. The GCxGC technique improved the separation of critical pairs such as damascenone and decanoic acid ethyl ester (Fig. 1). Fig. 2 shows the distribution of organic acids and their esters: they were grouped according to carbon number along the x-axis and by chemical class along the y-axis. The GCxGC enabled the separation of chemical classes such as cyclic aromatic compounds, sulfur and nitrogen compounds, linear and/or branched aliphatic compounds (Fig 3).



- Fig. 1: GCxGC trace of spelt beer. 1) 1,2-butandiol; 2) unknown; 3) 3-methyl butanediol; 4) 3-methyl butanol; acetate; 5) furfural; 6) 2-furanmethanol; 7) 3-methylthio-1-propanol; 8) heptanone; 9) ethyl acetate; 10) benzoic acid; 11) acetic acid benzyl ester; 12) phenyl-ethanol; 13) phenyl-ethanol; 14) ethylhexanoic acid ethyl ester; 15) octanol; 16) epi-pinene; 17) linalool; 18) acetic acid benzyl ester; 19) octanoic acid; 20) octanoic acid ethyl ester; 21) heptanol; 22) decanal; 23) phenyl ethyl acetate; 24) phenyl acetic acid 2-phenyl ethyl ester; 25) 4-vinylphenol-3-methoxy; 26) nerol; 27) nonanoic acid; 28) nonanoic acid ethyl ester; 29) decanoic acid; 30) 4-decanoic acid ethyl ester; 31) 1,10-dioxolane; 32) decanoic acid ethyl ester; 33) damascenone; 34) geranyl-lactone; 35) linalool; 36) dodecanoic acid; 37) unknown; 38) unknown; 39) humulone oxide.



Polar compounds, such as sulfur, aromatic and other heterocyclic compounds were more retained in the second dimension and were thus located in the upper part of the 2D plot with respect to the less polar compounds (aliphatic esters, alcohols, organic acids, ethers, hydrocarbons). The samples of spelt beers showed higher levels of aliphatic esters (ethyl hexanoate, ethyl octanoate, ethyl decanoate and ethyl dodecanoate) and lower contents of monoterpenes (linalool, terpineol, citronellol and nerol) and damascenone with respect to pils and double bock beers (Fig. 4 and 5). This profile was related to the PCA elaboration of the sensory analysis (Fig. 7) showing that pils and double bock beers were bitter, while the spelt beers had a fruity and honey flavour: this was presumably due to the typical fruity aroma of the ethyl esters.



## Conclusions

Comprehensive bidimensional gas-chromatography (2D-GC) coupled on-line with quadrupole mass spectrometry (qMS) was applied for the unambiguous characterization of the volatile profile of different beers sampled using DH-SPME. The column combination used in this study provided two almost independent separations resulting in structured and ordered chromatograms which are a powerful tool for the identification of unknown peaks. In fact, one main benefit of orthogonal GCxGC separations is that patterns formed by groups of molecules in the 2D plot are related to their chemical structure: this information in combination with mass spectra was employed for reliable peak identification in all samples. The spelt beers showed higher levels of aliphatic esters and lower contents of monoterpenes and damascenone with respect to pils and double bock beers. This profile was related to the sensory analysis showing that pils and double bock beers were bitter, while the spelt beers had a fruity and honey flavour due to the low content of bitter terpenes and high content of ethyl esters. The results of this study allow us to propose GCxGC/qMS as a promising technique for the characterization of volatile organic compounds of beer produced with different cereals. A better understanding of the key aroma compounds would be of significant importance for the modern brewing technology, particularly in the selection of raw materials, beer quality control and product development. In addition, some molecules may also be used as 'markers' in order to differentiate among samples of different commercial producers or fermentation conditions, aging and raw materials.

References: De Schutter D., D. Saison, Delvaux F., Derdelinckx G., Rockx J.M., Neven B., Delvaux F. Optimisation of wort volatile analysis by headspace solid-phase microextraction in combination with gas chromatography and mass spectrometry. J. Chromat. A, 1179, 2008; 75-80. Tian J., Yu J., Chen X., Zhang M. Determination and quantitative analysis of acetoin in beer with headspace sampling-gas chromatography. Food Chem. 2009; 112, 1079-1083. Saison D., De Schutter D., Delvaux F., Delvaux F. Optimisation of a complete method for the analysis of volatiles involved in the flavour stability of beer by solid-phase microextraction in combination with gas chromatography and mass spectrometry. J. Chromat. A, 1190, 2008; 342-349.

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