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Technical Report

Using Headspace SPME-GC×GC–quadMS for the Characterization of Marsala Wine

HS-SPME GC×GC–quadMS method for the analysis of Marsala wine

Flavio A. Franchina[1], Peter Q. Tranchida[1], Paola Dugo[1, 2], Luigi Mondello[1, 2]

Abstract:

The present contribution describes a research work focused on the elucidation of the composition of the headspace of Marsala wine. Samples were subjected to headspace solid-phase microextraction-comprehensive 2D GC analysis. At the outlet of the second GC dimension, the eluting analytes were split between a flame ionisation detector (for relative quantification purposes) and a rapid-scanning quadrupole mass spectrometer (for compound identification). The results attained open a door on the highly complex nature of the Marsala headspace.

Keywords: comprehensive 2D GC, quadrupole mass spectrometry, SPME, Marsala wine

1. Introduction

Marsala wine, or simply "Marsala", is a well-known, highly appreciated and economically important dessert wine, produced exclusively in Sicily. Marsala is exported all over the world and is considered one of the four most important dessert wines together with Madeira, Sherry and Port. Marsala is classified by the degree of ageing and sugar content. Considering the former characteristic, Marsala can be: "fine" (above 1 year), "superiore" (above 2 years), "superior riserva" (above 4 years), "vergine" (above 5 years), and "stravecchio" (above 10 years). With regards to the concentration of reducing sugars, the groups are: "secco" (below 40 g/L), "semi-secco" (between 40 and 100 g/L), and "dolce" (above 100 g/L).

Currently, the analytical technique of choice for the untargeted elucidation of aroma profiles, in foods and drinks, is one-dimensional gas chromatography, hyphenated to a mass spectrometer. It is obvious that GC–MS approaches can give qualitative (and possibly quantitative) information, but nothing on the odour sensation generated by a specific analyte. However, one of the main problems related to the 1D GC analysis of aromas is that such samples are excessively complex for a single GC column. The main consequence of insufficient separation power is that, often, compounds co-elute at the column outlet. In the present report, a head-space (HS) SPME-GC×GC–FID/MS method was developed for the analysis of Marsala wine (Fig. 1). The FID trace was exploited for quantification purposes, while the MS data was used for identification.

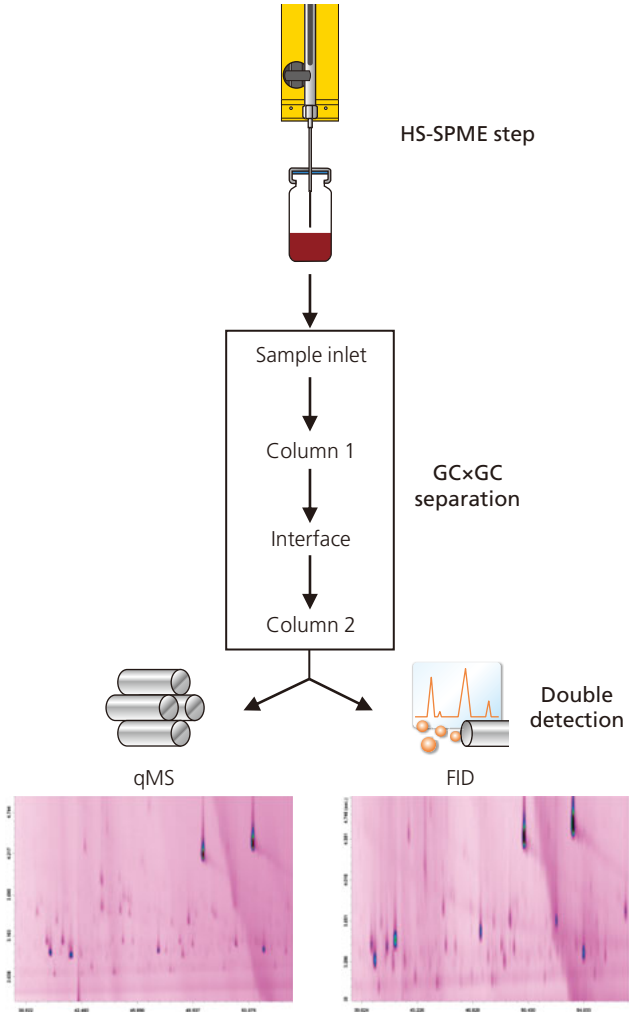

Fig. 1 Marsala wine analysis flow chart

1 University of Messina, Italy
2 Chromaleont S.r.l.

2. Experimental

Four types of Marsala, namely "fine", "superiore secco", "superiore riserva dolce", and "vergine", were donated by a producer located in Marsala (Sicily, Italy). The bottles were stored at ambient temperature, in the dark, prior to analysis.

A C_7–C_{30} alkane mixture, for linear retention index (LRI) calculations, was kindly supplied by Supelco (Milan, Italy).

Head-space SPME was performed using different SPME fibres (Supelco): triple phase divinylbenzene (DVB)/Carboxen (CAR)/polydimethylsiloxane (PDMS) (50/30 µm), PDMS (100 µm), CAR/PDMS (75 µm). A Shimadzu AOC-5000 autosampler (Kyoto, Japan) was used for the HS-SPME operations.

Four mL of Marsala were introduced in a 20-mL vial. The fibre was exposed in the headspace for 30 min, at ambient laboratory temperature (25–26 °C). During the extraction the vial was agitated (clockwise–anticlockwise alternate rotation) at 500 rpm. After this process, the fibre was thermally desorbed in the GC injection port for 1.0 min at 270 °C, in the splitless mode (after 1 min, a 10:1 split ratio was applied).

The Shimadzu GC×GC system consisted of:

- two GC-2010 gas chromatographs
- a GCMS-QP2010 Ultra quadrupole mass spectrometer
- AOC-5000 autoinjector
- a loop-type cryogenic modulator

Softwares used:

- GCMSsolution version 2.71
- GCsolution version 2.71
- ChromSquare version 2.0
- Shimadzu AFT launcher

D1 column: SLB-5ms (Supelco), 30 m × 0.25 mm ID × 0.25 µm d_f [silphenylene polymer virtually equivalent in polarity to poly (5% diphenyl/95% methylsiloxane)], + 1 m × 0.25 mm uncoated column to create a double-loop necessary for cryogenic modulation.

D2 column: Supelcowax-10 (Supelco), 1 m × 0.1 mm ID × 0.1 µm d_f and 1.5 m × 0.1 mm ID × 0.1 µm d_f (100% polyethylene glycol), connected to the FID and to the mass spectrometer, respectively. Inj. temp.: 270 °C; inj. press.: 150 kPa.

GC oven temp: 50 °C (hold 1 min) to 270 °C at 3 °C/min, (both ovens)

Modulation time: 5 sec.

MS parameters: full-scan mode (sampling frequency 25 Hz) with a scan speed of 10,000 amu/s (mass range of *m/z* 40–360); interface and ion source temperatures were 250 and 200 °C, respectively. MS ionization mode: electron ionization (70 eV).

FID parameters: temperature was 280 °C; acquisition frequency: 125 Hz; gases: make-up (He): 40 mL/min; H_2: 40 mL/ min; air: 400 mL/min.

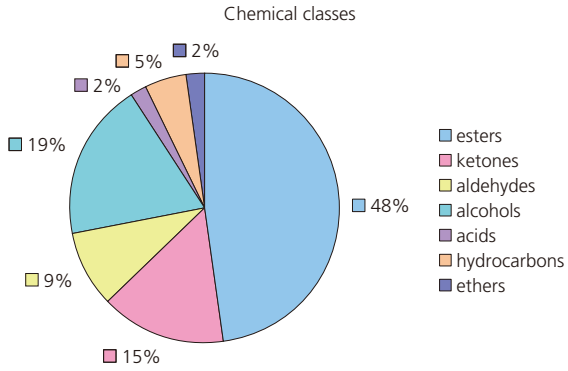

Fig. 2 Relative % peak areas for the main compounds found in Marsala.

3. Results and discussion

Initially, the SPME operation conditions were optimised considering fibre stationary phase, extraction time, and desorption time. With regards to the extraction temperature, no additional heating was used because the objective of the research was to provide a profile of Marsala volatiles, which is best performed at ambient temperature. Heating could potentially accelerate the extraction period, but would also alter the "normal" composition of Marsala headspace (Marsala is consumed at ambient temperature), and, consequently, the HS SPME-GC×GC fingerprint would not faithfully represent that of the Marsala volatile composition.

With regards to the fibre selectivity, the heterogenous composition (in terms of polarity) of Marsala headspace must be considered. In fact, Marsala volatiles range from acids, to alcohols, onto esters, aldehydes and ketones, down to hydrocarbons. The PDMS liquid polymer showed a poor coverage for the more polar volatiles (*i.e.*, alcohols, acids, aldehydes); on the other hand, the mixed DVB/ CAR/PDMS and CAR/PDMS fibres, which consist of a porous solid and liquid polymer, did not discriminate between low and high- polarity volatiles. However, because CAR is characterised by smaller pores with respect to DVB, the CAR/PDMS fibre gave an excellent performance towards the lower MW volatiles, and was much less selective towards the higher MW ones (> 200 amu). Overall, it was the DVB/CAR/PDMS phase that provided the best coverage in terms of polarity and analyte MW. The relative % composition of chemical classes in a Marsala wine analyzed is shown in Fig. 2.

After defining the most appropriate fibre, four different extraction periods were evaluated, namely 15, 30, 45, 60 and 75 min; as a good compromise, an extraction period of 30 min was chosen.

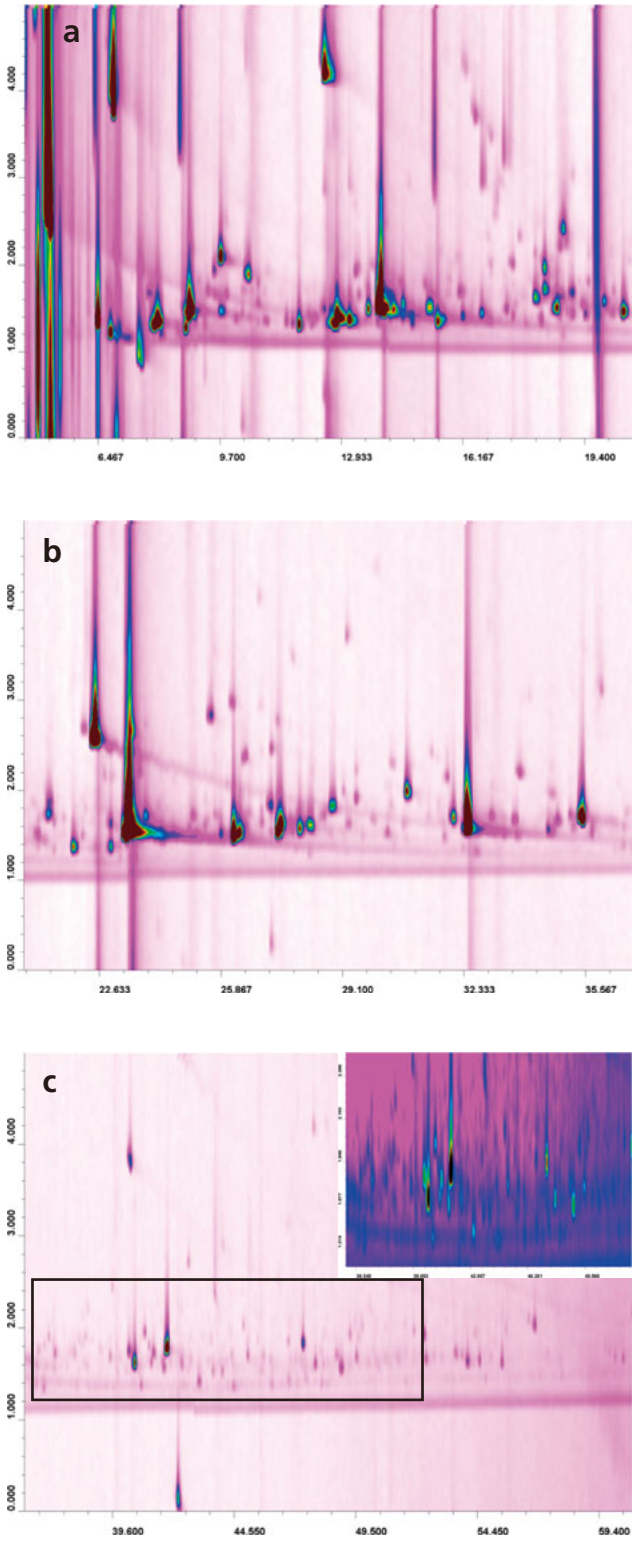

Fig. 3 a–c. Full-scan HS SPME-GC×GC–MS chromatogram expansion (initial, middle and final part) of Marsala "superiore secco". For peak identification *see* ref. 1.

With regards to the GC×GC–MS/FID method, a "classical" non-polar/polar column combination was employed. The column segments (*see* Experimental) connected to the detectors guaranteed a 50:50 split. The MS system was operated at 25 Hz and generated a sufficient number of data points/peak for quantification (at least 10). However, MS analyte response factors can vary greatly and so such instruments are generally not used to derive peak area % information. In this respect, FID response factors vary less, and hence such a detector can be exploited to attain such semi-quantitative data.

The three chromatogram expansions, reported in Fig. 3 a–c, illustrate the positions of the components identified in the "superiore secco" sample. Considering all four samples, 128 different compounds were tentatively identified. Ethanol (apart from water, the most abundant Marsala constituent) was not included as it obviously eluted within the MS solvent cut-off time. With regards to the other three samples, 87 compounds were identified in "fine", 91 in "superiore riserva", and 89 in "vergine". The first part of the chromatogram was characterized by the presence of abundant low molecular weight constituents (*i.e.* ethyl propanoate), poorly entrapped by the cryogenic modulator (*see* Fig. 3a).

The complex nature of the Marsala headspace is evident, with over 500 compounds detected. For space reason analyte identification table *see* ref. 1. Identification was performed through MS database searching, with the application of two filters: one based on a minimum spectral similarity value (75%), and the other on experimental/database linear retention indices [1].

Prior to discussion on quantification values, it must be noted that HS SPME-extracted analytes will give a good, but not an entirely faithful representation of the true headspace composition. In this respect, the FID% areas relate to the SPME fibre uptake. However, the data herein reported does give a good idea of the headspace composition.

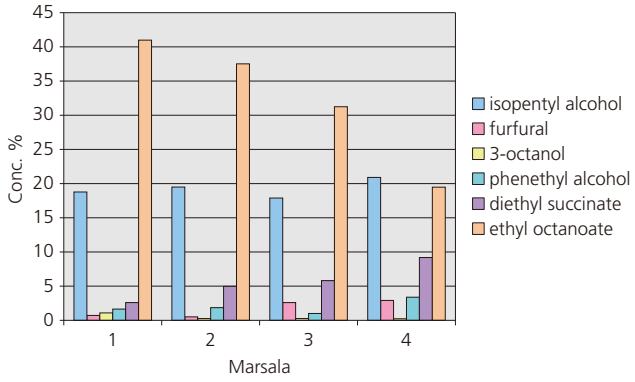

Fig. 4 Relative % peak areas for the main compounds found in Marsala (1= Fine; 2= Superiore Secco; 3= Superiore Riserva; 4= Vergine).

Prior to discussion on quantification values, it must be noted that HS SPME-extracted analytes will give a good, but not an entirely faithful representation of the true headspace composition. In this respect, the FID% areas relate to the SPME fibre uptake. However, the data herein reported does give a good idea of the headspace composition.

Fig. 4 lists a series of Marsala constituents, with an FID% area of over 1% in at least one of the samples. The value of 100% refers to the sum of the peak areas of the compounds identified (*e.g.*, 87 compounds were considered in Marsala fine). Intra-day precision (n = 3), for the analytes identified and for all four samples, was satisfactory with relative standard deviation values always lower than 20%.

The most abundant ester (and compound in general) was found to be ethyl octanoate, and decreased from the youngest to the oldest Marsala: 40.9%, 37.5%, 31.2%, and 19.4%.

Another ester, namely diethyl succinate, presented an opposite behaviour, inasmuch that the concentration increased from the youngest to the oldest Marsala: 2.7%, 4.9%, 5.7%, and 9.2%. The aldehyde furfural was found in much higher amounts in the "superior riserva" and "vergine" samples.

The most abundant alcohol (apart from ethanol) was found to be isopentyl alcohol, measured in comparable amounts in all sample types (17.9–21.0% range).

Considering the number of compounds detected, such an untargeted application could not have been achieved adequately by using a 1D GC process. Such a statement is fortified by observing the inset reported in Fig. 3b, which refers to the boxed area of the chromatogram, and has been extracted at a lower maximum signal intensity. The number of analytes visible in the inset are much higher compared to the boxed area, and gives a real picture of the complexity of the sample's volatile composition.

4. Conclusions

In conclusion, the HS SPME GC×GC–MS/FID method developed has opened a door on the aroma of Marsala wine. In general, the use of a GC–MS approach for the untargeted analysis of the volatile fraction of dessert wines (and wines in general) could be considered as an excessive analytical challenge. In principle, time-of-flight MS can spectrally "deconvolute" co-eluting solutes, at the GC outlet. However, the reliability of MS database identification does decrease, as the extent of overlapping increases. Consequently, GC×GC appears as the most suitable pre-separation method in such experiments.

Reference

[1] Dugo *et al.*, *Food Chem.* 142 (2014) 262–268.

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