InnOscent chromatographic system: An innovative device to revisit aroma analysis and recombination perspectives

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Abstract

Aroma results of a complex mixture of volatile compounds in which the contribution of each volatile depends on its concentration, detection threshold, interaction with the matrix and emergence of complex aroma-aroma interactions. [1] Gas chromatography (GC) coupled to olfactometry is useful to identify and estimate the individual potential of odour-active compounds, but it is irrelevant to predict the effective contribution of odorants to food aromas. Therefore, models reconstituted with chemicals are used to evaluate these contributions in a mixture context. [2] This approach requires time and above all, needs every compound to be identified, quantified, and commercially available. The InnOscent chromatographic device was designed to overcome these constraints and was configured to realize both conventional GC analysis coupled with multiple detectors, and innovative fraction collection experiments including recombination possibilities. This study aims at presenting this system through a wine aroma analysis.

An olfactometric analysis was first performed on a wine made of Cabernet Franc grape variety, with an 8 judges-panel and the InnOscent system configured to operate as a conventional GC coupled to a mass spectrometer (MS) and a dual olfactometric port. On the basis of the olfactometric results, recombined fractions of selected compounds were directly recovered from the system and were submitted to the panel in order to evaluate the contribution of these odour-active compounds to the wine aroma. 32 odorants were detected by at least 3 out of 8 judges in the wine aroma. Evaluation of the recombined extracts demonstrated that the mixture of all these odorants mimics the original wine aroma. Moreover, the mixture of the 14 most intense odorants was demonstrated to be representative of this aroma. The findings of this study illustrate the relevance of the system to realize a comprehensive aroma exploration using a single disposal. While freeing from chemicals, InnOscent system makes it possible to evaluate the contribution of any compound or group of compounds to an overall aroma, and thus go further in aroma analysis.

Introduction

Food aroma is a major criterion in consumers' appreciation prompting food producers and processors to regard it as a perennial issue. However, comprehension of aroma is still an ongoing scientific challenge since aroma results from a complex mixture of volatile compounds. All do not contribute equally to the aroma mainly due to their respective concentrations, detection thresholds, interactions with the matrix and emergence of complex aroma-aroma interactions. Gas chromatography (GC) coupled to olfactometry is a both analytical and sensory technique used to identify odour-active compounds and estimate their individual odorant potential. However, there is a gap between this individual characterization of compounds and their effective contribution to a food aroma. Thus, investigation of many product aromas turns to models that are reconstituted with chemicals, generally based on odour activity values. While functioning, this approach is time-consuming and requires every compound to be

B. Siegmund & E. Leitner (Eds): Flavour Sci., 2018, Verlag der Technischen Universität Graz
DOI: 10.3217/978-3-85125-593-5-101,

identified, quantified, and commercially synthesized. In this context, the InnOscent chromatographic system was designed to overcome these constraints and ease aroma comprehension. This device was configured to perform both (1) conventional analyses by GC coupled with a mass spectrometer, a single or dual olfactometric port, or a flame ionization detector, and (2) innovative fraction collection experiments including recombination possibilities. As a demonstration of the capabilities of this system, the aroma analysis of a wine made of Cabernet Franc grape variety was performed and the contribution of most potent odorants was investigated.

Experimental

Material

Wine used for the study was a red wine, 13% alcohol, from Bourgueil appellation, elaborated in 2010 from a Cabernet Franc vineyard. Chemical standards and n-alkanes were purchased from Sigma Aldrich (St Quentin Fallavier, France) with purity > 97%.

Wine aroma extraction

Volatiles from a 5mL wine sample were extracted by solid phase micro-extraction with a Car/PDMS fibre (10 mm length, 85µm film thickness; Supelco, Bellefonte, PA, USA) placed in the headspace of the vial for 10 minutes at 34°C after 1hour of incubation. Compounds were directly desorbed from the fibre in the injection port of the GC (T=260 °C).

Chromatographic device and conditions

Analyses were carried out with the InnOscent laboratory-designed system (Figure1) using an Agilent 7890A gas chromatograph combined with a 5975 mass spectrometer (MS, electron impact mode 70 eV, scan m/z 33-300, 2.7 scan.s^{-1}, Agilent Technologies, Wilmington, DE, USA). The column was a DB-Wax (Agilent, 30m length × 0.25 mm internal diameter × 0.5 µm film thickness), hydrogen was used as the carrier gas and oven temperature was programmed as follows: 50°C (0 min) to 80 °C at 5 °C·min^{-1}, then 80 to 200 °C at 10 °C·min^{-1}, and 200°C to 240 °C (4 min) at 20°C·min^{-1}.

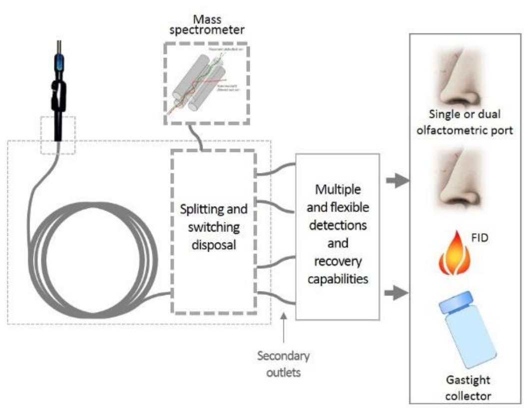

Figure 1: Schematic configuration of the InnOscent-GC device (patent pending) [3]

The end of the column was connected to a splitting and switching disposal allowing the eluate to be split and directed for one part to the MS and for the rest of the eluate towards secondary outlets. Throughout the run, the flow can be selectively transferred to one or another secondary outlet, *via* the events control module of Chemstation software (Agilent). Depending on the purpose of the experiments, secondary outlets can be connected to an olfactometric port (transfer line T=200°C, Gerstel ODP 3, Mülheim an der Ruhr, Germany), a flame ionization detector (FID, T=260°C, Agilent) or a collecting device.

Olfactometric analysis

The InnOscent system was first scheduled to get a conventional GC-MS coupled to a dual olfactometric port, connecting two olfactometric ports to secondary outlets. The eluate was analysed by an 8 trained judges-panel, throughout successive runs. Judges' perceptions (time, intensity and description) were recorded *via* the WheelOscent olfactometric software presenting an aroma wheel interface designed for wine analysis. [4] Results are displayed as an aromagram, directly obtained from the software, presenting the number of detections *vs* retention time. Identification of odorants was performed by comparing their linear retention index and mass spectra to those of databases (Wiley, Nist and internal databases) and by injection of standard compounds.

Recombination experiments and extracts evaluation

A collector was connected to a secondary outlet of the InnOscent GC-MS system and an FID was also connected to the system allowing to control the recovered extract. The total extracts were first recovered in the collector throughout successive runs. Then the recombined extracts were recovered. Switching events were programmed so that only selected compounds were directed to the collector and the FID, whereas other fractions of the eluate were eliminated through other secondary outlets. Extracts containing odorants perceived by at least, 3, 4, 6 and 8 judges on the basis of the olfactometric results were this way successively collected in different collectors. Timing of the switching events was set according to the GC-MS-O results. Collectors containing the recombined and total extracts were coded with a three-digit random number and submitted to the panel in a randomized order. The judges were asked to smell the content of the collectors and score the similarity of the odour with that of a total extract named reference. Anova was performed on similarity scores obtained for each extract with a 95% confidence level.

Results and discussion

The aromagram and chromatogram of the wine aroma obtained from the InnOscent device present different patterns, underlining that compounds with the most abundant peak areas are not necessarily the most frequently perceived, and highlighting that instrumental and human detections are complementary. Thirty-three compounds were perceived by at least 3 out of the 8 judges (Figure) and 25 compounds were identified. Among them a majority of ethyl esters and acetates are found, as well as alcohols, acids, carbonyl and sulfur compounds, phenols and pyrazines. These observations are consistent with extensive literature dealing with wine aroma analysis. [5]

On the basis of these olfactometric results, recombined extract were directly recovered from the InnOscent device and were submitted to the panel to evaluate their contribution to the aroma. The extract that contained the 33 compounds detected by at least 3 out 8 judges, was perceived as representative of the wine aroma with a similarity score of 7.7 out of 10 (Table 1). This is particularly true considering the similarity score (8.2 out of 10) given to the total extract compared to the identical reference, explained by the natural reluctance of the judges to use the ends of the scale. This result illustrates the capability of the olfactometry analysis to point out the compounds involved in the wine aroma.

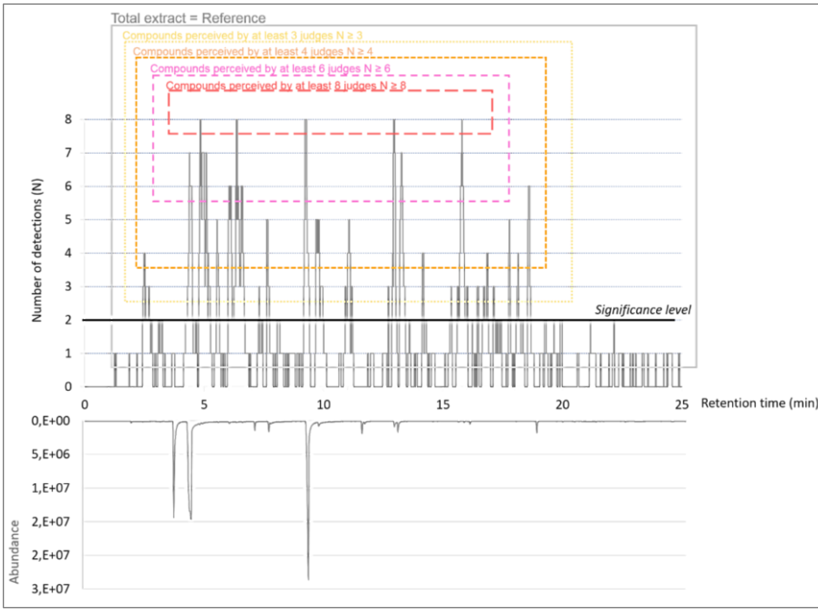

Figure 2: Aromagram and MS chromatogram of the wine aroma.

Table 1: Similarity scores obtained from the 8 judges-panel for the different extracts recovered directly from the InnOscent GC device compared to a total extract.

	Recombined extract with x compounds perceived by at least :				*Reference*
	8 judges x= 5	*6 judges x= 13*	*4 judges x= 24*	*3 judges x= 33*	*= total extract*
Similarity score	2.2[b]	7.2[a]	7.4[a]	7.7[a]	8.2[a]

Furthermore, if the mixture of the 5 more perceived compounds did not allow to reconstitute the wine aroma, the mixture of the 13 more frequently perceived compounds demonstrated to be sufficient to produce an extract perceived as representative for the wine aroma. This study illustrates the possibilities given by the InnOscent device combining conventional analysis and innovative omission/recombination capabilities. The system provides solutions to directly evaluate the aroma of mixture of compounds or estimate the contribution of any target compound to a global aroma, overcoming constraints of current approaches. This approach will deliver valuable information to understand complex aroma-aroma interactions and to go further into aroma comprehension.

References

1. Guichard, E. (2002) Food Rev. Int. 18: 49-70.
2. Ferreira V. Ortin N., Escuerdo A., Ricardo L., and Cacho J. (2002) J. Agric. Food Chem. 50: 4048–4054.
3. Villière A., Lethuaut L., Prost C. and Ecole Nationale Vétérinaire, (2013) WO 2013140057 A1 also published as EP 2828652 A1 and EP 2828652 B1.
4. Villière A., Le Roy S., Fillonneau C., Guillet F., Falquerho H., Boussely S. and Prost C. (2015) Flavour 4: 24.
5. Fischer U. (2007) In Flavours and Fragrances (Berger, P., ed.) Springer B.H., pp.241-267.