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Producing headspace extracts for the gas chromatography–olfactometric evaluation of wine aroma

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ABSTRACT

In this work different designs of headspace devices were studied. Using these designs, 23 volatile compounds of wine aroma added to a synthetic wine were purged by an inert gas (nitrogen) and trapped in a solid-phase extraction (SPE) cartridge. Efficiency was measured as the similarity between the odour intensity in a model wine in the glass and the odour intensity recorded in the gas chromatography–olfactometry (GC–O) experiment.

Using the initial design, the polar compounds were not detected in olfactometry from solutions in which the measured orthonasal intensity was intermediate; these compounds were undervalued by this technique. New headspace device designs were then tried. The least amount of under-valuation was obtained when the purging distance between the sample and the trapping system was reduced, which suggests that polar molecules were being trapped by polar surfaces in the original design. The improvement in detection was general, affecting all chemical compounds, resulting in overvaluation of non-polar compounds. A strong correlation of log *P* with the under-valuation/overvaluation of compounds was noted.

From the comparison of headspace devices, one design was found which efficiently transferred even the high-polarity compounds from the wine to the trap. Therefore, this system is recommended for obtaining extracts of aged red wine for use in a screening system based on olfactometry.

1. Introduction

Gas chromatography–olfactometry (GC–O) is a powerful tool for the study of chemical compounds responsible for wine aroma. However, what is quantified by the GC–O process is quite distinct, from both qualitative and quantitative points of view, from the perception experienced during actual wine consumption. Wine flavour is often very complex, composed of many volatile and non-volatile chemical entities. What one perceives in the glass is a mixture of odour and taste molecules which may combine to act in a suppressive or additive manner, or synergistically (Frijters & Schifferstein, 1994).

In GC–O, by contrast, the odorants are delivered to the olfactory epithelia as single entities, which simplifies the recognition task. Furthermore, the compounds are fully volatilised and there are no other substances present which could retain compounds or influence their perception. Therefore, the quantity of compound delivered to the olfactory epithelia, as well as the perception reported, depends critically on the isolation technique used.

Aromagrams depend on the assay method, particularly on the extraction step (solvent and solventless methods). The extracts used for olfactometric hierarchising of odorants in any given product can vary widely, depending on the isolation system used (de Koning, Janssen, & Brinkman, 2009; Plutowska & Wardencki, 2007; Sides, Robards, & Helliwell, 2000) and/or the concentration factor as it relates to the initial product. The extract must represent the characteristics of the product to be studied (Plutowska & Wardencki, 2008; Priser, Etievant, Nicklaus, & Brun, 1997). Also, it is important to choose the most appropriate method of olfactometric analysis. If one uses a dilution method (Ferreira, Pet'ka, & Aznar, 2002), the concentration of the initial extract is not as important as when one uses a frequency detection method (Pollien et al., 1997) or a method of estimating intensity (Priser et al., 1997), in which the concentration of the extract is crucial for avoiding saturated signals (Ferreira, Pet'ka, Aznar, & Cacho, 2003).

Several studies (Campo, Cacho, & Ferreira, 2008; Campo, Ferreira, Escudero, & Cacho, 2005; Campo, Ferreira, Escudero, Marqués, & Cacho, 2006; Escudero, Campo, Farina, Cacho, & Ferreira, 2007) have shown that the recorded GC–O signal (labelled "modified frequency" or MF in these studies) takes into account both the evaluation of intensity and the frequency of detection of an odorant. Using the formula proposed by Dravnieks (1985), MF is the geometric mean of the detection frequency of an aromatic zone (ex-

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pressed as a percentage) and the average intensity (expressed as a percentage of the maximum intensity). The quantitative ability of this technique has already been proved (Ferreira, Pet'ka, Aznar, & Cacho, 2003). In wine aroma studies, MF is a useful concept because, in general, a large number of odorants exist in concentrations near the threshold, and also because members of the tasting panel can have widely divergent sensitivities.

The initial objective of the present study was to investigate the compatibility of GC–O results with those obtained from wine-tasting, specifically the orthonasal perception of odour intensity in model wine.

A further aim of the present study was to develop a highly efficient, fast and simple screening method of analysis, based on purge and trap, headspace-solid-phase extraction (HS-SPE) coupled to GC–olfactometry, for studying many of the volatiles that compose the aroma of wine, and to calibrate that method.

2. Material and methods

2.1. Reagents and standards

Dichloromethane of HPLC quality was obtained from Fisher Scientific (Loughborough, UK), methanol of LiChrosolv quality was from Merck (Darmstadt, Germany), absolute ethanol (ACS quality), tartaric acid (ACS quality) and sodium hydroxide (ACS quality) were purchased from Panreac (Barcelona, Spain), and pure water was obtained from a Milli-Q purification system (Millipore, Billerica, MA). *Resins*: Polypropylene cartridges and LiChrolut EN resins were purchased from Merck (Darmstadt, Germany).

Chemical compounds used in system calibration: these were representative of different chemical families present in the aroma of wine and are shown in Table 1. The chemical standards were supplied by Aldrich (Gillingham, UK), Fluka (Buchs, Switzerland), Merck (Darmstadt, Germany), Lancaster (Strasbourg, France) and SAFC (St. Louis, MO) (Table 1).

Synthetic wine: this hydroalcoholic solution was prepared by dissolving tartaric acid (5 gL^{-1}) in a water–ethanol mixture (12% v/v ethanol) and adjusting the pH with a sodium hydroxide solution to pH 3.4.

Real wines: initial and final designs were tested with two real wines: a red wine of 14% v/v ethanol, variety Mencía from D.O. Bierzo, vintage 2004 (wine 1); and another red wine of 13.2% v/v ethanol, variety Graciano from D.O.C. Rioja, vintage 2004 (wine 2).

2.2. System evaluation

2.2.1. Orthonasal intensity determination

The panel of sensory experts was composed of five females and one male between the ages of 24 and 40; all were laboratory staff members. The tests were carried out with normalised tulip glasses containing 20 mL of synthetic wine. Twenty-three odour-active compounds were chosen as examples of chemical groups relevant to flavour research (Table 1). The panellists received training to familiarise them with the odour of each compound. In this first session, judges were asked to rank, according to perceived orthonasal intensity, four glasses with different concentrations (0, 10, 100 and 1000-times higher than its odour threshold) of each of the studied compounds in synthetic wine. Following the initial training period, various synthetic wines were prepared, containing each molecule to be studied in varying concentrations. Orthonasal intensity was evaluated in order to discover what concentration of each volatile compound gave an intermediate odour intensity of 1.5 on a 7-point scale (0 = no odour; 1 = weak odour, low intensity; 2 = clear perception of odour, strong intensity; 3 = extremely strong intensity of odour; intermediate values of 0.5, 1.5, and 2.5 were allowed). For this purpose, two reference glasses were prepared. One of them contained synthetic wine without addition of the studied compound ($I = 0$), and the other contained a sufficient concentration of the same compound to provide a maximum orthonasal intensity ($I = 3$). Panellists evaluated the orthonasal intensity of a solution

Table 1
Suppliers, thresholds, concentrations and orthonasal intensity of studied compounds.

	Supplier	Odour threshold (μg/L)	Concentration in synthetic wine (mgL^{-1})	Orthonasal intensity in synthetic wine	Error[a]
Ethyl 2-methylbutyrate	Fluka	18[c]	1.4	1.3	0.23
2-Methylpropanol (isobutanol)	Merck	40000[c]	750	1.4	0.18
Ethyl lactate	Aldrich	155000[c]	1550	2.0	0.10
3-(Methylthio)propanal (methional)	Aldrich	0.5[b]	0.025	1.3	0.17
Decanal	Aldrich	10[c]	0.43	1.5	0.14
Linalool	Aldrich	25[c]	4.4	1.2	0.18
3-(Methylthio)propanol (methionol)	Aldrich	1000[c]	50	1.3	0.18
γ-Octalactone	Aldrich	7[d]	42.9	1.2	0.11
2,5-Dimethyl-4-hydroxy-3(2H)-furanone (furaneol)	Fluka	5[c]	0.25	1.4	0.16
4 Allyl-2-methoxyphenol (eugenol)	Fluka	6[c]	2.6	1.6	0.27
4,5-Dimethyl-3-hydroxy-2-(5H)-furanone (sotolon)	SAFC	0.7[c]	0.07	2.0	0.16
2,4,6-Trichlorophenol	Aldrich	0.35[b]	3.5	1.6	0.14
2,3,6-Trichlorophenol	Aldrich	0.35[b]	3.5	1.5	0.17
Indole	Aldrich	50[b]	5.0	1.7	0.21
Skatole	Aldrich	50[b]	0.5	1.3	0.23
2,4,5-Trichlorophenol	Aldrich	0.35[b]	5.0	1.7	0.16
Methyl vanillate	Aldrich	3000[c]	379	1.4	0.34
o-Cresol	Aldrich	31[c]	3.1	1.6	0.08
m-Cresol	Aldrich	68[c]	6.8	1.3	0.25
p-Cresol	Aldrich	30[b]	3	1.6	0.35
4-Ethylguaiacol	Aldrich	33[c]	3.3	1.8	0.11
3-Ethylphenol	Aldrich	0.5[b]	0.05	1.7	0.33
4-Ethylphenol	Aldrich	35[b]	3.5	1.7	0.17

[a] Standard mean error ($s/n^{1/2}$; $n = 6$).
[b] Thresholds calculated in the laboratory in a 12% water/ethanol mixture at pH 3.4.
[c] This value has been taken from bibliography Cullere, Escudero, and Cacho (2004).
[d] This value has been taken from bibliography Ferreira, Jarauta, Ortega, and Cacho (2004).