Gas Chromatography Olfactometry

Subjects: Chemistry, Analytical

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Gas Chromatography-olfactometry (GC-O) is, by nature, the technique of choice for the screening of the molecules with odour (odorants) responsible for the aromatic sensory properties of any product. Ideally, the GC-O technique should provide an unbiased ranking of the odorants attending to the relevance of their contribution to those sensory properties. Such ranking is essential for further steps directed to the elucidation of the chemical nature of the odorants, for their quantification or for a basic understanding of the chemical bases of the aromatic perception in such product. The review discusses the different approaches for GC-O specifically applied to deciphering wine aroma. The critical difference between approaches is whether the ranking of odorants is carried out on an extract containing all the odorants present in the product or on an extract representative of the odorants contained in the vapour phases that cause the odour and flavor. Historically, most researchers have preferred techniques based on total extracts, because of sensitivity and operational issues. It is argued that the second alternative is more direct and can be more efficient, but it requires a good understanding of the factors affecting orthonasal olfaction, handling volatiles (purging, trapping, eluting, and separating) and about the sensory assessment of GC effluents.

odorants odours flavour gas chromatography-olfactometry sensobolome

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1. Wine Aroma

Wine is a very special food product whose value is increasingly associated with the set of characteristics, both extrinsic and intrinsic, responsible for the pleasure associated with its consumption. Extrinsic elements such as connections with geography and history, brand image, or perception of exclusivity, amplify the pleasure associated with the purely sensory perceptions, which are the intrinsic and primary elements of wine quality [1]. Within these sensory perceptions, complexity and aromatic balance are two key elements [2][3]. It should be remarked that the most appreciated wines rarely have explicit and easy to define aromas, rather they have complex aromatic notes in which some fruit and freshness perception is essential, along with other spicy, woody or toasted notes, depending on the type of wine. Note that, especially for experts, the absence of aromatic defects or deviations is also always an essential element of quality [4].

The set of wine aromatic perceptions includes all the different odours perceived through the nose during wine consumption. These odours change with time due to the progressive evaporation of the most volatile compounds once the wine is poured in the glass [5][6], changing both orthonasal and retronasal perceptions. Behind those odours there are several dozens of wine odorants able to reach our olfactory epithelia during wine consumption. The set of perceived olfactory perceptions are the result of various processes of modulation and integration of the primary olfactory signals produced by each one of the odorants. In the in-mouth perceptions, integration includes stimuli from the senses of taste and touch. All these integration processes make it difficult to understand the relationship between the primary olfactory inputs and the perceived aroma. For instance, cooperative associations between very weak odorants of more or less similar odours can produce clear and net odours [7][8], or the strong suppression effects of some components such as 2,4,6-trichloroanisole (TCA) or higher alcohols [9] can completely suppress other relevant odours. The corollary is that understanding wine odour characteristics requires more than just studying its most intense odorants.

Some odorants are common to all wines and can be considered “constitutive” of wine. Among them, the secondary volatile metabolites of alcoholic fermentation, or in the case of oak aged wines, the wood extractable volatiles. Most of these “constitutive” volatiles are also relatively easy quantified by GC-MS since they are in affordable concentration ranges (several µg-mg/L). There is, however, a second group of relatively common odorants, many of which derive from the grape, which can be found in much wider concentration ranges. Terpenes, norisoprenoids, volatile phenols, vanillins, rotundone, methoxypyrazines or polyfunctional mercaptans are found in this group. Some of them are responsible for the specific aromatic properties of certain types of wine. The low concentrations at which they can become active can complicate the analytical control, particularly in the cases of polyfunctional mercaptans, methoxypyrazines or rotundone. Something similar happens to some potential off-odours, such as TCA and other halophenols, or 1-octen-3-one, *E*-2-nonenal and other fatty acid-derived odorants. The list of potentially relevant aroma compounds, both positive and negative, increases steadily with time. This is in part the logical consequence of our scientific and technical progress, but unfortunately, and particularly for negative aroma compounds, such increase is a side consequence of the increasingly frequent anomalous climatological phenomena affecting grape maturation.

2. Gas Chromatography-Olfactometry as a Technique for Screening Odour-Active Molecules

Gas chromatography-olfactometry (GC-O) has been used almost since the introduction of gas chromatography, as the human nose is the most appropriate detector to monitor the presence of an odorant in the effluent of a gas chromatograph [10]. For GC-O, the flow at the outlet of the chromatographic column is divided into two branches by means of a union or Y-joint, one that carries the analytes to an instrumental detector (FID, MS,.); and another one that takes them to an olfactometric port, where the human nose acts as a detector of great sensitivity and selectivity. The first forms of GC-O consisted simply on the sensory description of the effluent from the chromatographic column with the aim of assessing whether the chromatographic peak was odour active. In the case of grapes and wines, the first reports date from the 70′s, when the technique was first used to identify C6 alcohols and aldehydes as responsible of the leafy odour of grape leaves [11] and to monitor changes in aroma composition during aging [12]. One of its first successes was the identification of furaneol as key off-odour of the wines made with *V. labrusca* hybrids [13].

The potential of GC-O as a screening technique able to rank the odorants present in a product attending to their potential relevance in the product begun to be recognized in the 80′s with the pioneer works of Acree, et al. [14] and Schieberle and Grosch [15]. These authors introduced the two first systematic approaches for obtaining quantitative parameters related to the olfactory importance of an odorant in a given product: charm analysis and AEDA, respectively. Charm is the acronym for Combined Hedonic Aroma Response Measurements and AEDA for Aroma Extract Dilution Analysis. The techniques will be later presented and discussed with more detail. Now, some previous disquisitions about the goal of the GC-O screening operation will be elaborated to clarify some concepts which often are not correctly understood by researchers.

The obvious goal of the GC-O screening operation is to rank the odorants present in the product attending to their relative implication on the aroma-related sensory properties of the product. For this, the shortest way is to carry out the GC-O screening operation on an extract whose composition closely resembles those of the vapour phases emanating from the product during its olfaction and/or consumption. However, producing such an extract is not straightforward at present, and it was yet more complicated 30 years ago. By then, early researchers realized that the direct GC-O study of headspaces (usually carried out under equilibrium conditions) yielded just a very little fraction of the most volatile odorants present in the product, which at the end, resulted to be not really much important on its odour and flavour [16][17]. Those headspace fractions were also so diluted that identification was very difficult. Because of these reasons, most researchers decided to get a “total extract” from the product, and even today, the GC-O operation is most often carried out on such total extract after the corresponding operations of cleaning and concentration. A “total extract” can be easily obtained from any product. For that, the product just has to be extracted with relatively high volumes of a solvent of medium polarity (diethyl ether or dichloromethane), preferably using several consecutive extractions. This type of extracts can easily contain 100% of the odorants present in the original product, and from this point of view, they are “representative” of the product. However, it is of the outmost importance to understand that these types of extract cannot provide unbiased estimations of the relative importance of the different odorants in the sensory properties of the product. The reason for this has to do with the fact that in GC, all the volatile components introduced in the chromatographic column end volatilized and reach the detector, regardless of their volatility. On the contrary, in the original product the different odorants are transferred to the vapour phases at very different proportions, depending on their specific volatilities in the product matrix. These volatilities do not depend only on the size and boiling point of the odorant, but on the interactions that it establishes with the matrix. Unfortunately, these volatilities in aqueous matrixes can be so different between odorants that can completely invalidate the ranking obtained in the GC-O operation carried out on the total extract. To illustrate this situation let’s take as example two similarly powerful odorants with very different polarities: vanillin and 2,4,6-trichloroanisol (TCA), whose properties are summarized in Table 1.

Table 1.—Basic chemophysical properties and odour thresholds of vanillin and 2,4,6-trichloroanisol (TCA), two powerful odorants of very different polarities.

Property	Vanillin	2,4,6-Trichloroanisole (TCA)
Molecular weight (g mol^{-1})	152.2	211.5

Boiling point (°C)	285	241
Log P	0.59	4.11
Water solubility (mg L^{-1})	6875	10
Henry's volatility constant (atm L mol^{-1} at 25 °C)	$2.5\ 10^{-9}$	$1.3\ 10^{-4}$
Log Koa	8.3	6.4
Odour threshold in air (µg L^{-1})	0.008	0.004
Odour threshold in water (µg L^{-1})	100	0.00003

As can be seen, both odorants have very similar odour thresholds in air, which indicates that they are similarly powerful, i.e., our noses require similar numbers of molecules of both components to elicit a detectable odour signal. However, their odour thresholds in water differ by more than 6 orders of magnitude. This difference is due to the different polarities of both molecules. While TCA is quite hydrophobic and scarcely soluble in water (log P = 4.1, Wsol = 10 mg L^{-1}), vanillin is quite hydrophilic and water soluble (log P = 0.59, Wsol = 6.9 g L^{-1}). The volatility from aqueous solutions, is given by the Henry's volatility constant, and as can be seen, that of TCA is more than 5 orders of magnitude higher than that of vanillin, which basically tells us that TCA is more than 5 orders of magnitude more easily transferred from an aqueous solution to the vapour phase, which explains its much lower odour threshold in water. Let's recall, however, what will be the outcome of a GC-O experiment carried out on a "total extract" obtained from an aqueous product in which both compounds are present at 1 µg L^{-1}. As both components will be equally extracted, the GC-O operation will tell us that both odorants are equally important in the original product. The truth, however, is that TCA is 300 times above threshold, while vanillin is 100 times below. This example should let us conclude that any GC-O screening operation carried out on a "total extract" most likely provides a biased hierarchy of odorants. The odorants more retained (less volatile) in the original matrix will be highly over-estimated. In aqueous and hydroalcoholic matrixes, this will happen to all the polar and water-soluble odorants (acids, alcohols, phenols, mercaptans…).

Aware of this bias, the most widely used and accepted GC-O screening strategy, originally proposed by Schieberle and Grosch [15], also known as "sensomic" or "molecular science concept", includes as part of the screening strategy the experimental determination of so-called odour activity values (OAVs, quotients concentration/odour threshold) of all the odorants identified in the GC-O screening. Once the concentration of the odorant is corrected by its odour threshold in the product matrix, the volatility differences responsible for the bias of the olfactometric screening become corrected, so that the OAV list provides an un-biased hierarchy of the odorants in the product. I.e., in this strategy the ranking provided by the GC-O screening is simply an intermediate operation whose goal is to identify the molecules with odour in the product but cannot anticipate their role on the sensory properties.

Experience has demonstrated that the “molecular science concept” works. However, it can be argued that it is time consuming and quite inefficient, since all odorants found in the total extract have to be identified and quantified, while only a little fraction are relevant. Any strategy providing extracts for GC-O representative not of the product, but of the vapour phases emanated from the product, should make it possible to make an earlier selection of the “a priori” most relevant odorants, saving much work. This requires to overcome the difficulties of obtaining headspace fractions fulfilling the following two requirements:

- To be concentrated enough to detect and identify all relevant odorant of the product
- To be truly representative of the vapour phases emanated from the product

These two conditions are nowadays affordable. The comparison between both philosophies, with some of their advantages and disadvantages are summarized in Table 2.

Table 2. The two different general approaches to GC-O screening operation.

Questions	Total-Extract Based	Representative Headspace-Extract Based
Goal. What do we rank in the GC-O screening operation?	All the odorants present in the product, regardless of differences in transference rates to vapour phases	The odorants responsible for the odours and flavours elicited by the product
Emphasis	The odorants in the product	The odorants in the vapour phases emanated from the product
Extract. What should it contain?	All the odorants present in the product (at 100%)	The odorants present in the vapour phases emanated from the product
Result. What have we ranked?	Odorants attending to their olfactory importance in the extract	Odorants attending to their olfactory importance in the vapour phases

How results of the GC-O relate to the aroma-related sensory properties of the product?	Poorly. Olfactometric scores overemphasize the importance of the odorants more retained in the food matrix. A valid hierarchy is obtained only after OAV determination	If the extract is really representative of product headspaces, olfactometric scores should be closely related to aroma-related sensory properties of the product
Disadvantages/difficulties	Too much work. The hierarchy only will emerge after all OAVs have been estimated (all odorants have to be identified and quantified)	It is difficult to ensure that the extract is really representative of the vapor phases. Some odorants can be at too low levels in the extract for identification and quantification (a more concentrated extract may be necessary)
Global assessment	Excruciatingly long but trustful	Economical and efficient if a good and representative headspace extract is obtained

To the best of our knowledge, the above classification is proposed for the first time. In general, researchers tend to name and classify the GC-O screening operation attending to the specific olfactometric strategy followed (for instance AEDA, NIF, posterior intensity or Osme). However, the olfactometric strategy is secondary, since it rather affects to the how, while the key definitory parameter of the GC-O is its goal, which defines the what. Keeping in mind these two different possibilities, the two main elements of a GC-O screening operation, namely obtaining the extract and the GC-O strategy, will be briefly discussed.

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