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Review

Methods for gas chromatography-olfactometry: a review

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Abstract

Gas chromatography-olfactometry methods are used in flavor research to determine the odor active compounds in foods. In this review, the four major methods for gas chromatography-olfactometry are described and their potentials and limitations discussed. The methods include dilution analysis, detection frequency methods, posterior intensity methods and time-intensity methods. The value of gas chromatography olfactometry data is shown to depend directly on the gas chromatography-olfactometry method, as well as on sample preparation and analytical conditions. Each of the methods has been used frequently and has its advantages and disadvantages. However, on the methodological side, a considerable area is still to be explored, which would contribute to the interpretation of the data and would improve the value of these techniques for both fundamental and applied research. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Aroma; Flavor; Gas chromatography-olfactometry; Volatile compounds

1. Introduction

Progress in analysis techniques over the last decades has led to long lists of volatiles determined in foods [1]. Some of these volatile compounds contribute to the odor of a food and some to the aroma of a food. Odor perception can be considered the response to odor active volatiles that enter through the nostrils (orthonasal) and aroma perception is the perception resulting from volatiles that enter from the mouth and respiratory system (retronasal) [2]. The perception of volatile compounds released from foods by the human nose depends on the extent of release from the food matrix and on the odor properties of the compound. There are indications that only a small fraction of the large number of volatiles occurring in food actually contributes to the odor and aroma [3]. Therefore, the distinction between odor active compounds and the whole range of volatiles present in a particular food product is an important task in flavor analysis. An interesting approach is sniffing the gas chromatographic effluent of a representative isolate of volatile compounds of a food, in order to associate odor activity with the eluting compounds. Many of the 'chemical' detectors are not as sensitive as the human nose for many odor active compounds [4]. Experience shows that many odor active compounds occur at very low concentrations; their sensory relevance is due to low odor thresholds. Therefore, the peak profile obtained by any 'chemical' detector does not necessarily reflect the aroma profile of a food [5]. Gas chromatography-olfactometry (GC-O) was proposed by Fuller et al. as early as 1964 [6] and has shown to be a valuable method for the selection of odor active compounds from a complex mixture [7]. With the early GC-O devices, reproducibility was a serious problem, which was caused by discomfort from sniffing hot dry effluent gases and the lack of sensitivity of the 'chemical detectors' to the odor active compounds. The latter problem is still with us today. Dravnieks and O'Donnell [8] published a GC-O design in 1971, which minimized the discomfort of sniffing. The hot GC effluent was combined with humidified air to reduce nasal dehydration. Nowadays, the same principle is still used in most GC-O apparatuses. In general, it is very difficult to judge the sensory relevance of volatiles from a single GC-O run. Initially, volatiles were sniffed individually

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when eluting from a GC column and a description of the odor was given for each retention time, corresponding to an odor active compound [9]. GC-O is limited to this screening for odor active volatile compounds, unless any quantification of the chemical stimuli and of the assessors' responses is performed. It should, of course, be kept in mind that in GC-O, single compounds are assessed. This approach does not provide information on their behavior in a mixture, although it indicates the relevance of some compounds for the aroma of a food. Recombination of odor active compounds in the food matrix to match the original aroma of the food and subsequent sensory evaluation can be used to prove the correct selection of odor active compounds as a final step in aroma analysis. In addition, correlations between the odor active compounds present and the sensory data of food products also indicate the relevance of the compounds.

The purpose of this paper is to review recent developments in GC-O analysis techniques, including those methods that have not received much attention in reviews before. Classic and newer GC-O methods will be compared and their potentials and limitations discussed.

2. GC-O methods

Several techniques have been developed to collect and process GC-O data and to estimate the sensory contribution of single odor active compounds, which can be classified in four categories [4].

1. Dilution analysis methods for producing potency values based on stepwise dilution to threshold, e.g. combined hedonic response measurement (CharmAnalysis) [10,11] and aroma extraction dilution analysis (AEDA; [12]).
2. Detection frequency methods for recording detected odors over a group of assessors. The number of assessors detecting an odor (detection frequency) is used as an estimate of the odor's intensity [13].
3. Posterior intensity methods for producing estimates of perceived intensity, which are recorded after a peak has eluted [14].
4. Time-intensity methods for producing estimates of perceived intensity recorded simultaneously with the elution of the chromatographic peak, e.g. Osme [15].

2.1. Dilution analysis

Initially, sniffing experiments were combined with traditional threshold analysis to give a value called the aroma value [16]. This value was defined as the ratio of the concentration of an odor active compound to its odor threshold. Other groups have used this ratio with various methods of threshold determination to give values that include the odor unit number [17], the number of odor intensity units [18], the odor value [19], the odor intensity index [20], the flavor unit [21] and the threshold odor number [22]. Some groups have used GC-O to determine the thresholds of compounds and to relate them to the concentration in the food product, similarly to odor unit numbers. For example, Ferreira et al. [23] determined the GC-O detection limits of compounds in order to study odor unit values of compounds in hydroalcoholic solutions. Berger et al. [24] reported the determination of the 'best estimate-GC-lower amount detected by sniffing' (BE-GC-LOADS) and used these values for similar purposes.

The technique of (extract) dilution sniffing analysis has been developed by two different research groups [10,12], in an effort to simplify the method used for determining a unit of odor intensity. The aim of the technique is to determine the relative odor potency of compounds present in an extract. The method gives the priority order for chemical identification and adds to the understanding of the chemical origins of olfactory differences [7].

In dilution analysis, an extract is diluted, usually as a series of 1:2 or 1:3 dilutions, and each dilution is sniffed until no significant odor is detected. Several injections are required to reach a dilution of the aroma extract in which odorous regions are no longer detected. Both CharmAnalysis and AEDA are based on this odor–detection threshold principle. In AEDA, the dilution factor (FD value) is simply the last dilution at which an odor active compound is detected. The results are usually presented as the logarithm of the factor of dilution (log FD) versus the retention index or by listing the FD values [25]. An example of an AEDA aromagram is presented in Fig. 1, showing a reference mix of eight volatile compounds differing in concentration for one assessor. The method has been used to determine the

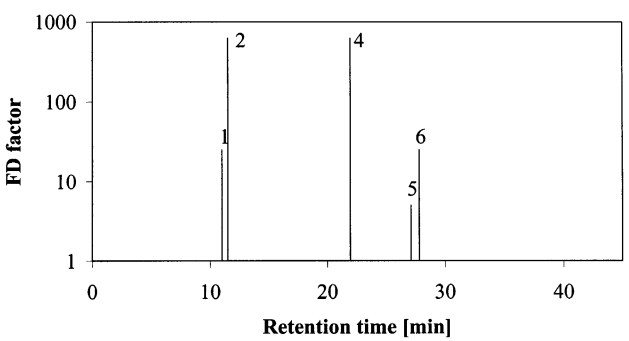

Fig. 1. Aroma extract dilution analysis aromagram of eight volatile compounds in a reference mix conducted by assessor HW: 100 ng 2-butanone (1), 20 ng diacetyl (2), 500 ng ethyl acetate (3), 100 ng 3-methyl-1-butanol (4), 20 ng ethyl butyrate (5), 100 ng hexanal (6), 100 ng 2-heptanone (7) and 500 ng α-pinene (8). Compounds 3, 7 and 8 could not be detected by assessor HW.

potent odor active compounds in many different food products, including roasted beef [7], roasted coffee [26], tea [27], popcorn [28], wheat and rye bread [29], chicken broth [30], white wines [31], soybean oil [32], cod and trout [33] and in model systems [34–36]. More recently, Guth and Grosch [37] reported a new concept of AEDA using static headspace instead of extracts. Dilution steps were made by injecting decreasing headspace volumes to evaluate the contribution of a compound to the whole composition.

Acree et al. [10] developed a dilution analysis technique called CharmAnalysis, that is also based on the sniffing of decreasing serial dilutions of volatiles. In CharmAnalysis, the dilutions are presented in randomized order to avoid bias introduced by knowledge of the samples. The assessor points out the beginning and end of each particular odor perception (duration of the smell) with a sensorial descriptor. Times of the individual sniffs are combined and graphed to yield a chromatogram with peaks and quantified peak areas (Charm values), which are used to quantify potency. A Charm value can be calculated according to the formula $c = d^{n-1}$ where n is the number of coincident responses and d is the dilution factor [38]. CharmAnalysis has been frequently used for determination of potency of odor active compounds in foods, such as cherry juices [38], apples [11,39,40], orange juice [41], grapes [42] and in a commercial methyl jasmonate sample [11] for glucose–proline reaction products [43].

The difference between AEDA and CharmAnalysis is that in CharmAnalysis the duration of perception is taken into account together with the final dilution (dilution value) in which a compound is detected. This dilution value is analogous to the factor of dilution (FD value) in AEDA. In fact, the dilution value at the peak maximum in a Charm chromatogram is identical to the FD factor calculated when the data are plotted on an AEDA basis [7].

The task of the sniffing assessor in dilution analysis is relatively simple and sniffing successive dilutions of the same extract gives validation of the final result obtained from the multiple detection of the same odor. Nevertheless, a major drawback of the dilution approach is the difficulty of using more than one assessor, as is advisable in sensory analysis, because the method is very time-consuming. Another drawback is that in dilution analysis, the compounds that are perceived at the highest dilution level are deemed the most potent in the sample. With these methods, the results are proportional to the odor unit number first defined by Rothe and Thomas [16]. It is assumed that the response to an odor stimulus is linear with the dilution and that all compounds have identical response slopes with increasing concentration. The concept of odor unit number as a measure of the relative intensity of odor active compounds in an extract has been largely criticized by Frijters [44]. The odor unit number defines the relation between two physical quantities. According to Frijters, it does not specify the relationship between a physical and a perceptual measure and is, therefore, not a psychophysical concept. The odor unit number is based on two assumptions that are contrary to present psychophysical theories of odor perception. The use of the odor unit number assumes that there is a linear relationship between the perceived intensity of a compound and its concentration, which has been proven to be invalid by both Fechner's and Steven's laws [45]. These laws show a logarithmic or power relationship between the odor intensity and the physical concentration. Similar relationships between the intensity and stimulus have been reported for taste [46], texture [47] and loudness [48]. The second assumption is that the slopes of perceived intensity versus concentration are equal for all odor active compounds. However, many authors have reported different slopes for different compounds [49–53]. The fact that different odor active compounds do not necessarily have equal intensity/concentration slopes puts serious doubts on the merits of the odor unit number as a measure for 'relative contribution' to the intensity of a mixture. An increase with a particular multiple of threshold concentration units of the concentration of two compounds having equal thresholds (same FD values or dilution factors), may result in drastic intensity differences in the non-diluted extract and vice versa. Therefore, a rank order of compounds on odor unit numbers (aroma values) does not necessarily correspond to a rank order of perceived intensities.

2.2. Detection frequency methods

Detection frequency methods overcome the limitations of a small number of assessors and the use of detection thresholds. The method proposed by Linssen et al. [13] uses a group of assessors instead of one or two assessors. The number of assessors detecting an odor active compound at the sniff port simultaneously (the frequency of detection) is used as a measure for the intensity of a compound. A sniffing chromatogram can be composed which cumulates the number of detections of the compound. Usually, the effluent is split for two sniff ports and a flame ionization detector. Thus, two assessors sniff the effluent simultaneously. One analysis, using a panel of ten assessors requires five identical gas chromatographic runs. An example of a sniffing chromatogram for the eight compounds of a reference mix for eight assessors is shown in Fig. 2. A dummy sample has often been used to determine the signal-to-noise level of the group of assessors [54]. Pollien et al. [55] have reported a similar technique based on the frequency of detection. The detection frequency method has been evaluated for various quantities of aroma

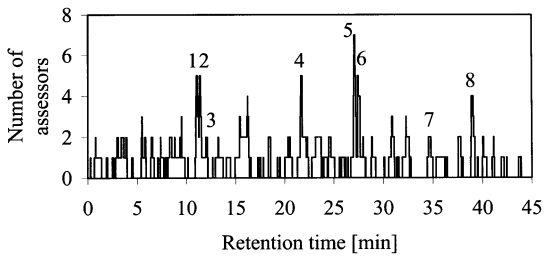

Fig. 2. Sniffing chromatogram of eight volatile compounds in a reference mix obtained by the detection frequency method using a panel of eight assessors. Numbers refer to compounds in Fig. 1.

compounds. Different sampling times (1–12 min) in a model mouth system resulted in various quantities of compounds and numbers of assessors detecting the compounds. However, the different sampling times gave an identical selection of odor active compounds (signal above noise level of the group of assessors), which showed the robustness of the method [54]. The method has been used for selection of odor active compounds in many foods, i.e. bell peppers [56–59], dried French beans [47,56,60], dried leeks [56], chocolate [61], vegetable oils and emulsions [62,63], cheese [64], mineral water [13,65], coffee [66], lovage [67] and packaging materials [68–70]. Significant correlations have been established between the number of assessors perceiving odor active compounds correlated and intensity scores of attributes in sensory analysis [47,56,60,71]. Furthermore, the number of assessors perceiving odor active compounds were shown to relate very well to the intensity of an odor active compound, recorded after elution from the column [47,54,72]. Despite good correlations between numbers of assessors and intensities at the sniff port and intensities of sensory attributes for a number of compounds, it is a drawback that the method is not based on real intensities.

2.3. Posterior intensity methods

The posterior intensity method involves the recording of the odor intensity on a scale after a peak has eluted from the column. The method has not been reported in the literature frequently. Validation of the techniques, with respect to its relationship with physical concentrations of compounds and with other methods, has not received much attention. Only recently, van Ruth et al. [72] showed linear relationships between the logarithm of the stimulus at the sniff port and the average posterior intensity score of a panel of eight assessors. Large variability was observed between the assessors. In Fig. 3, an example of intensity scores is given for the eight volatile compounds in the reference mix for a panel of eight assessors and for the single assessor, whose data were used for AEDA in the previous section on dilution analysis (Section 2.1). Variance among assessors was considerable. The rank orders of the eight volatile compounds of the reference mix in odor potency/intensity for AEDA and detection frequency method (data presented in Figs. 1 and 2, respectively) and posterior intensity method are shown in Table 1. The data resulting from the posterior intensity method correlate reasonably well with those of the detection frequency method ($R = 0.822$). Lower correlation coefficients were obtained for posterior intensity and dilution analyses ($R = 0.667$). The few references on this method include Drawert and Christoph [73], who reported the influence of peak width and height on odor intensity scores, which were based on an estimate of one person. The method has also been applied in studies on apple flavors [14]. Furthermore, the posterior intensity method was used to evaluate the flavor of orange juice [74], Cheddar cheese aroma [75], odor active compounds of light-activated milk [76] and dried French beans [47].

The task for the assessor is moderately complicated in the posterior intensity method. Use of the scale differs considerably among assessors. To reduce variation, the end of the scales could be anchored with references in theory. However, it is practically impossible to provide a reference during a GC-O run.

2.4. Time-intensity methods

Time-intensity methods are based on magnitude estimation of the odor intensity. McDaniel et al. developed a time-intensity method called Osme [15,77–81]. Trained assessor(s) directly recorded the intensity and duration of each odor active compound detected at the sniff port and described the odor perceived. They used a variable resistor with a pointer moving along a 16-point category scale. A simultaneous computerized graphical feedback of the settled position of the cursor helped the assessor to adjust this position to the perceived intensity. A panel of four assessors was used to determine relationships between odor intensities and

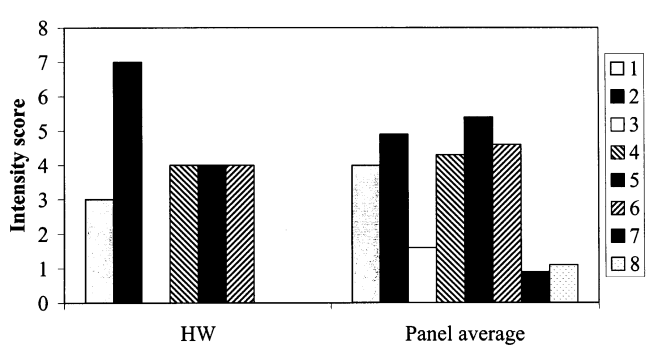

Fig. 3. Intensity scores of eight volatile compounds in a reference mix for assessor HW and the panel average ($n = 8$). Numbers refer to compounds in Fig. 1.

Table 1
Comparison of GC-O techniques for ranking odor potency/intensity of a reference mix of eight volatile compounds

	Dilution analysis[a]	Detection frequency[b]	Posterior intensity[c]
2-Butanone	3.5	3	5
Diacetyl	1.5	3	2
Ethyl acetate	7	6.5	6
3-Methyl-1-butanol	1.5	5	4
Ethyl butyrate	5	3	1
Hexanal	3.5	1	3
2-Heptanone	7	8	8
α-Pinene	7	6.5	7

[a] Ranking based on dilution factors (FD values) of assessor HW.
[b] Ranking based on numbers of assessors detecting an odor, panel of eight assessors including assessor HW.
[c] Ranking based on average intensity scores of a panel of eight assessors, including assessor HW.

concentration. Both the maximum odor intensity of the compounds and the area under the odor intensity peak showed significant correlations with the physical concentration of the compounds in the GC effluent [77]. Variation between assessors showed the importance of the use of a panel. Delahunty et al. [82] and Guichard et al. [83] reported a time-intensity method using a computer mouse along a scale and Guichard et al. [83] and Étiévant et al. [84] reported a cross-modality matching method with the finger span based on the same principle. They described a prototype for the precise measurement and acquisition of the distance between the thumb and another finger during analysis. Their four-member panel was able to determine most characteristics of the solutions with reference compounds and to create a finger span multidimensional space highly correlated with the theoretical intensity space. However, they also showed relatively poor individual performance of the assessors and they recommend the use of several individuals to perform this type of analysis.

Despite its promising approach, there have not been many applications of the Osme technique reported so far. One study on wine aroma has been published [80], however, the developers of the Osme technique used the frequency of detection, which is a form of the detection frequency method discussed in one of the previous sections, instead of the actual intensities. The same group has published Osme intensities of aroma compounds present in apples [81]. Furthermore, a study on cheese flavor has been published [82] in which the method was used to select volatile compounds contributing to the flavor of the cheese.

Currently, the time-intensity methods have not been used very frequently for GC-O. Methodological aspects should receive more attention before the value of this technique can be fully evaluated. For instance, it is unknown how reproducible the technique is and how parameters of time-intensity generally relate to physical concentration and to posterior intensity measurements.

3. General considerations

3.1. Aroma/odor isolation technique

In order to relate aroma/odor composition of a food to its sensory properties, the isolate or extract used for GC-O should represent the aroma/odor composition expressed when foods are eaten or smelled. Representative isolation of volatiles includes both qualitative and quantitative aspects. Extracts of foods usually represent the composition of the volatile compounds present in a food, whereas headspace isolates represent the composition of the volatiles present in the air above a food. For extraction of volatile compounds from a food, heat treatment should be limited to avoid formation of artifacts and decomposition of volatile compounds. In some cases, scientists have used a well-defined isolation procedure and compared the relative differences between samples, e.g. in comparative AEDA [85,86]. Factors influencing volatile release in the mouth include breakdown of the food matrix through mastication, which might lead to generation of specific compounds by endogenous enzymes in the food. The physical form of food changes during consumption due to hydration and dilution by saliva. These factors create significant differences between the classical headspace volatile profile and the actual profile in the mouth and nose during eating. Model mouth systems have been developed, as well as breath-by-breath analysis, to overcome these differences. Several authors have recently reviewed these volatile isolation techniques, including the influence of the type of isolation and the volatile profile obtained [87–90]. In addition, distillation, extraction and headspace methods were compared by GC-O using various food products, such as meaty/savory flavorings [5,91], bell peppers [92], wine [93,94] and beer [95]. Each of these papers showed a large influence of the isolation method. When one considers that the number of odor active compounds detected by GC-O depends on the isolation method, which includes variables often arbi-

trarily selected, such as amount of food sample, concentration factor, sample volume injected, it is obvious that the method for isolation of volatile compounds as well as all the variables must be chosen with care.

3.2. Analytical conditions

When samples are prepared for GC-O, it has to be taken into account that some volatile compounds are labile and occur in very low concentrations. Long storage periods of isolates should therefore be avoided. Furthermore, some unstable volatiles readily decompose in heated injector blocks and form artifacts. Sulfur-containing compounds are particularly susceptible to heat-induced decomposition. Chromatographic behavior of compounds varies with the compounds and the stationary phases of the GC column and might affect GC-O data. The only two studies found in literature focused on these phenomena showed that broader peaks resulted in higher thresholds [5] and lower intensities [73]. The odor character of some compounds depends on their concentration (e.g. skatol), which might become important in the situation of poor chromatography (peak broadening) or poor chromatographic separation (co-elution) of odor active compounds.

3.3. Assessors in GC-O analysis

In GC-O, assessors judge the olfactory impressions elicited by the volatile compounds immediately after elution from a GC column. Methodological problems may arise from the non-random sequence in which the compounds elute. Not all judgments are similarly affected by variation in the quality of the responses during an experimental session. Results of a GC-O experiment can be systematically affected by decreasing alertness. Decrease in alertness will be most important when only a small number of compounds can be perceived, when these compounds show low odor intensity, when the stimulus is brief, when a session is long and when assessors are not motivated [96]. Sensory and cognitive transfer effects can also affect consecutive judgments. Furthermore, problems can arise due to the varying inter-stimulus intervals, sometimes assessors have to make decisions very rapidly [97]. Therefore, it is not surprising that many authors showed large variability within and between assessors. Acree et al. [4,98] showed a considerable variance in Charm values for individual assessors as well as between assessors. Similar results have been published by Etiévant et al. [84] for time-intensity methods. It can be concluded from the studies of these various authors, that a group of assessors is a prerequisite for reliable GC-O analysis.

4. Concluding remarks

GC-O techniques have been applied frequently. However, some light on the methodological aspects of GC-O would improve the value of these techniques for both fundamental and applied research. Queries are still with us today with respect to the relationship between, e.g. determined parameters, such as thresholds and intensities, and between time-intensity measurements and posterior intensity scores. Related to this matter, the effect of chromatographic parameters, such as the peak shape of the eluting compound (high and narrow or low and broad) on the intensity of a compound is unknown. Finally, the relationship between odor activity of single compounds and their behavior in a mixture, as is usually the case with foods, has received limited attention from flavor chemists.

The four methods — dilution analysis, detection frequency method, posterior intensity method and time-intensity methods — have shown their value. Each of these methods has been used frequently to determine odor active compounds. However, the methodological side is still a considerable area to be explored.

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