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Evaluation of two gas chromatography–olfactometry methods: the detection frequency and perceived intensity method

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Abstract

Two gas chromatography–olfactometry methods were evaluated in terms of repeatability, range of sensitivity and discriminating properties. Six volatile flavour compounds at various concentration levels were analysed by a panel of eight assessors using the detection frequency method and the perceived intensity method. The coefficient of variance, averaged over the individual compounds for three replicate samples, was 16% for the detection frequency method and 28% for the intensity method. The average correlation coefficient of the individual compounds with concentration was 0.93 (range 0.88–0.99) for the intensities. They were slightly higher than those for the detection frequencies (0.91, range 0.81–0.97). The detection frequency method was more accurate in terms of repeatability, and the intensity method was more accurate with regard to discrimination between concentration levels. The range of sensitivity was similar for both methods.

Keywords: Aroma; Detection frequency; Gas chromatography–olfactometry; Odour intensity; Food analysis

1. Introduction

Combining human perception of odour and chromatographic separation of compounds, i.e. gas chromatography–olfactometry (GC–O), offers great possibilities. Applications include correlation of sensory responses with volatile compounds, resolving off-flavour problems, and assessing olfactory acuity of individuals. The chromatographic separation of compounds can be a difficult task, which depends mainly on the complexity of the flavour. However, the registration and quantification of the sensory perception are also two of the main challenges of the technique.

Most methods aim to rank the volatile flavour compounds detected in order of sensory importance. The three main types of methods used are the dilution to detection threshold method, the detection frequency method, and the perceived intensity method. In dilution analysis an extract is diluted, and each dilution is sniffed until there are no longer any odours detected. The last dilution at which a compound is detected is a measure for its odour potency [1,2]. The detection frequency method [3–5] uses a group of assessors. The number of assessors detecting an odour in the GC effluent simultaneously (detection frequency) is a measure for the sensory importance of a compound. Usually a group of 6–12 assessors assess a specific sample. The third group of GC–O techniques are intensity methods, which measure the odour intensity of a compound in the GC effluent. This group of methods encompass the posterior intensity method [6], the cross-modality matching finger span technique [7], and time-intensity methods [8].

The assessment of the importance of volatile compounds during GC–O analysis is based on sequential presentation of the flavour compounds in the mixture. The technique can introduce skewed ranking for a number of reasons. First, there is the use of assessors. The number of judges used in GC–O has to be considered since it has been demonstrated that individuals can perform very differently due to differences in thresholds and response criteria [9]. Differences in thresholds have been attributed to the age of the assessor, flow rate of the stimuli and experience with the experimental procedures [10]. Training [11] and breathing frequency [12] have also been reported to affect GC–O performance of individuals. Most of the problems with individuals can be overcome by using a group of assessors as was thoroughly studied by Pollien et al. [4]. Apart from the influence of the method used and the aspects directly related to the assessors,

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analytical conditions such as peak width [9,11] and the humidity of the effluent [11,13] sniffed affect GC–O results.

Although GC–O is widely used as a tool for attribution of sensory importance to specific volatile compounds, the analytical performance has received less attention. In the present study, the aim was to evaluate two GC–O methods in terms of repeatability, range of sensitivity, and discrimination between different concentrations. The two methods concerned the detection frequency method and the perceived intensity method. Six volatile flavour compounds at various concentration levels covering the dynamic range of the GC in the chosen set-up, were analysed by a panel of eight assessors.

2. Experimental

2.1. Chemicals

A solution of six volatile flavour compounds in pentane (Sigma-Aldrich, Steinheim, Germany) was prepared (Mix A) and consisted of: 2-butanone (12.5 mg ml^{-1}; Sigma-Aldrich), ethyl acetate (62.5 mg ml^{-1}; Sigma-Aldrich), diacetyl (2.5 mg ml^{-1}; Sigma-Aldrich), ethyl butyrate (2.5 mg ml^{-1}; Merck-Schuchard, Hohenbrunn, Germany), hexanal (12.5 mg ml^{-1}; Sigma-Aldrich), and α-pinene (62.5 mg ml^{-1}; Sigma-Aldrich). Five serial dilutions (1:5) of the solution were prepared by stepwise dilution with pentane. To simplify presentation of the results, the original solution is considered to have a concentration of 1, and the other concentrations were calculated relative to the original solution. For determining the repeatability of the methods the same solution was prepared, but slightly different concentrations were used: all flavour compounds were present at 1 mg ml^{-1} (Mix B).

2.2. Instrumental analysis

For GC–O, an aliquot (0.4 μl) of the reference solution or one of its dilutions was injected on Tenax TA (SGE, Kiln Farm Milton Keynes, UK). Thermal desorption of the volatiles from Tenax was performed by a thermal desorption device (225 °C, 5 min; SGE concentrator/headspace analysis injector, Kiln Farm Milton Keynes). Cryogenic focusing was applied on the analytical column (SGE CTS.LCO2, Kiln Farm Milton Keynes) to reduce band broadening. Gas chromatography was carried out on a Varian Star 3400 CX (JVA Analytical Ltd., Dublin, Ireland) equipped with a BPX5 capillary column (60 m length, 0.32 mm i.d. and 1.0 μm film thickness; SGE, Kiln Farm Milton Keynes). Helium gas (200 kPa) was used as carrier gas. An initial oven temperature of 40 °C was used for 4 min, followed by a rate of 2 °C min^{-1} to 90 °C, then by 4 °C min^{-1} to 130 °C, and finally by 8 °C min^{-1} to 250 °C. At the end of the capillary column the effluent was split 80:10:10 for the flame ionisation detector (FID; 275 °C), sniff port 1 and sniff port 2, respectively. FID responses confirmed consistency of the injections and sample preparation for replicates (average coefficient of variance of replicates <10%).

Eight assessors (women, aged 30–50) experienced in sensory analysis were selected on their sensitivity, memory, availability and ability to recognise odours. Prior to sniffing the dilutions, the assessors were trained on the technique of sniffing with mixtures of the same compounds described above, which varied in concentrations as well as on other samples. Assessors used laptop computers with a program in Pascal for data collection [3]. They pressed a key on the keyboard when they detected an odour, and pressed it again when the odour had disappeared. The data were converted from the field disks into Excel software in order to process the raw data. Assessors rated the perceived intensities of the eluting compounds on a 9-points intensity interval scale (1 = extremely weak, 9 = extremely strong) after their odour detection.

The samples were analysed in random order. Tenax tubes without absorbed volatile compounds were used as dummy samples for determining the signal-to-noise level of the group of assessors.

2.3. Statistical evaluation

Panel average intensity scores were calculated for the various compounds and concentrations. The increase of detection frequency and intensity as a function of concentration was calculated (the slope) and correlated (Pearson's product moment correlation coefficients: r [14]). To compare the two methods, the slopes of the various compounds from the two methods were correlated similarly. A significance level of 5% was used throughout the study.

3. Results and discussion

3.1. Repeatability

GC–O analysis was carried out on a mixture of 2-butanone, ethyl acetate, diacetyl, ethyl butyrate, hexanal and α-pinene in pentane (Mix B) using detection frequency and perceived intensity methodology. The compounds varied in odour quality and possessed the following odours: 2-butanone ethereal, ethyl acetate pineapple, diacetyl buttery, ethyl butyrate fruity, hexanal grassy, and α-pinene pine-like [15,16]. To examine the repeatability of the methods, the mixture was analysed in triplicate using both methods and eight assessors. The concentration was chosen, based on preliminary experiments, to ascertain that the sample was not at the end of the dynamic range, but that on the other hand as many assessors as possible would detect the compounds.

The average results for the group of assessors are displayed in Tables 1 and 2. The six compounds varied in detection frequencies and intensities. Generally, the variance

Table 1
Detection frequencies of six volatile flavour compounds in mix B determined by gas chromatography–olfactometry analysis using detection frequency methodology and a group of eight assessors

Compound	Rep 1	Rep 2	Rep 3	Average
2-Butanone	1	2	2	1.7 ± 0.6
Ethyl acetate	3	2	3	2.7 ± 0.6
Diacetyl	6	6	7	6.3 ± 0.6
Ethyl butyrate	7	6	6	6.3 ± 0.6
Hexanal	7	7	8	7.3 ± 0.6
α-Pinene	5	6	5	5.3 ± 0.6

for both detection frequencies (CV = 16%) and intensities (CV = 28%) are acceptable for this type of analysis. The data agree with results of Chaintreau et al. [17], who reported also larger variance for intensity than for detection frequency measurements. However, the range of variance among the compounds is larger for the detection frequencies (9–35%) than for the intensities (20–38%). This may originate from the fact that some compounds are more easily picked up than others. This in turn might be due to the fact that the concentration of the compound is close to the threshold of some individuals, or the nature of some odours may make it hard to distinguish them from the background. A negative correlation between the detection frequencies and the coefficients of variance of the various compounds was observed ($r = -0.95$). This generally implies that the coefficient of variance went down with higher detection frequencies. However, it should be kept in mind that the detection frequencies never differed more than one between the replicates for all the compounds. This shows the robustness of the method. The intensities showed only slight negative correlation with the coefficients of variance ($r = -0.70$). Ferreira et al. [18] demonstrated that the standard deviation tends to be smaller for compounds with a high intensity. Despite the differences in variance, the average results in terms of odour strength obtained by the two methods correlated well ($r = 0.99$).

The repeatability of individuals for the intensity measures was generally poor (average CV over compounds and individuals = 69%). These results are in agreement with studies of Chaintreau et al. [17]. These authors reported high coefficients of variance (69–144%) for repetitions of individuals with regard to the analysis of a model flavour mixture by GC–O, but much lower variance (ca. 25%) when the average of the panel was considered (here 16%).

Table 2
Intensities of six volatile flavour compounds in mix B determined by gas chromatography–olfactometry analysis using a group of eight assessors

Compound	Rep 1	Rep 2	Rep 3	Average
2-Butanone	0.4	0.4	0.6	0.5 ± 0.1
Ethyl acetate	0.9	1.9	1.3	1.3 ± 0.5
Diacetyl	4.4	3.0	3.4	3.6 ± 0.7
Ethyl butyrate	2.6	4.9	3.6	3.7 ± 1.1
Hexanal	3.1	3.4	4.5	3.7 ± 0.7
α-Pinene	2.1	2.5	3.6	2.8 ± 0.8

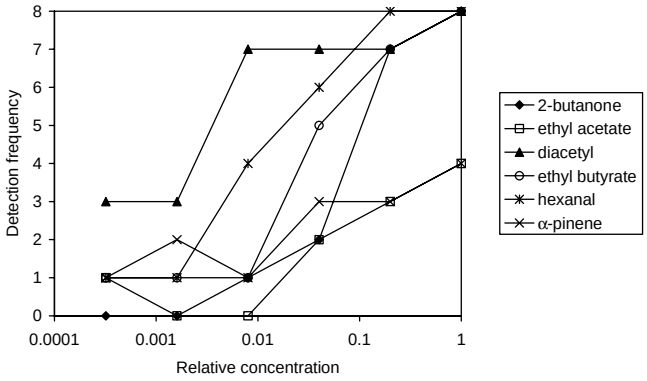

Fig. 1. Detection frequencies of six volatile flavour compounds in mix A at six concentration levels using gas chromatography–olfactometry analysis and a group of eight assessors.

3.2. Range of sensitivity and discrimination between concentration levels

In the second part of the experiments six concentrations of the mix A, which was composed of the six volatile flavour compounds, were analysed by GC–O using the detection frequency and the perceived intensity method. After preliminary experiments, the concentration range was chosen to cover the whole dynamic range of the GC, from below threshold to the point overloading occurred. Overloading was related to either the Tenax trap, the analytical column or the assessors. The concentration range that could be analysed covered ca. three magnitudes. Except for hexanal, most compounds reached their maximum detection frequency with the highest concentration level (Fig. 1). This implies that the maximum number of assessors was not a limiting factor for use of the method in the present set-up. The intensities (Fig. 2) did not show significant levelling off at the higher concentrations. However, it should be considered that at all concentrations a number of assessors were not able to detect

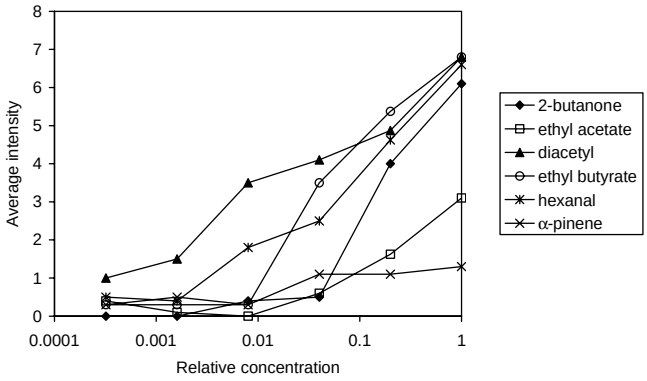

Fig. 2. Average intensities of six volatile flavour compounds in mix A at six concentration levels using gas chromatography–olfactometry analysis and a group of eight assessors.

Table 3
The slopes and Pearson's product moment correlation coefficients (r) for the detection frequencies and intensities of six volatile flavour compounds in mix A vs. the logarithm of the relative concentration[a]

Compound	Detection frequency		Intensity	
	Slope	r	Slope	r
2-Butanone	3.2 (6)	0.95	2.3 (5)	0.92
Ethyl acetate	1.6 (3)	0.97	1.1 (2)	0.93
Diacetyl	1.4 (2)	0.81	1.7 (3)	0.98
Ethyl butyrate	2.9 (5)	0.96	2.6 (6)	0.97
Hexanal	2.6 (4)	0.96	2.2 (4)	0.99
α-Pinene	0.9 (1)	0.83	0.3 (1)	0.88
Average		0.91		0.93

Ranks of slopes in order of increasing steepness in brackets.
[a] Relative concentration range (0.0016–1).

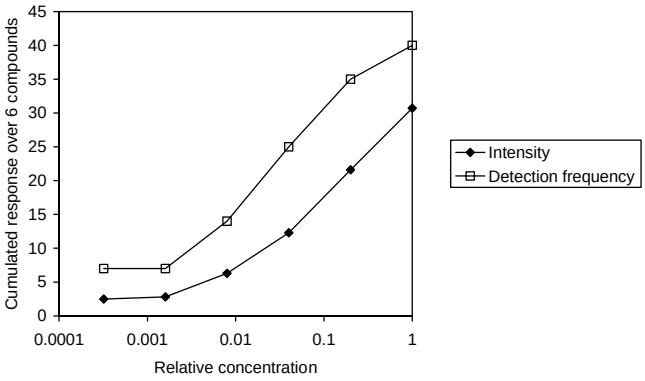

Fig. 3. Cumulated responses for detection frequencies and intensities over six volatile flavour compounds in mix A at six concentration levels using gas chromatography–olfactometry analysis and a group of eight assessors.

some compounds, despite the fact that the concentrations at the highest level varied from 500–2500 ng at each sniff port. These are high concentrations for headspace food samples. Taking into account that subsequent dilutions were analysed till all compounds were below noise level, the concentrations presently measured covered the normally analysed concentrations in food flavour samples. Thus, in terms of range of sensitivity, both the detection frequency and the perceived intensity method gave satisfactory results.

In order to compare the change in detection frequency and intensity with concentration for the different compounds, their log linear relationships (slopes and Pearson's correlation coefficients) were calculated (Table 3). Log linear relationships showed significantly higher correlation coefficients than power and linear functions ($P < 0.05$). High correlation coefficients were determined, ranging from 0.81 to 0.99. These results are in agreement with Fechner's law. This law describes psychophysical functions, which relate chemical concentration to perceived intensity [19]. Other studies showed sigmoidal functions as well [20,21], although some found that Stevens law or the more recently proposed relationship based on Hill's model fits [22] better. The compounds 2-butanone, ethyl butyrate and hexanal showed the steepest slopes, both for detection frequency and intensity. α-Pinene demonstrated the lowest slope value. The differences in slopes can be explained by differences in psychophysical functions as was shown before for other compounds [20,21]. Low slope values may imply that thresholds for these compounds are spread over a larger concentration range. Alternatively, the shape of the peaks may play a role. Relatively broad peaks, such as α-pinene, tend to result in lower intensities than expected for their overall quantity in the effluent [9,11]. The slopes of the compounds for the two methods correlated well ($r = 0.91$).

The results of the repeatability experiments showed that detection frequencies for all compounds differed never more than one between replicates. In addition, sniffing of blanks showed that the signal-to-noise level of the group of assessor was also one. Therefore, a difference in detection frequency of two or more (>twice the max and average standard deviation) is considered as cut-off point here to examine the discriminatory property of the method for these compounds at various concentration levels. Comparing the successive concentrations of the individual compounds, it was observed that seven out of 20 detection frequencies above noise level differed two or more. Nine out of the 20 intensities differed more than twice the standard deviation for the individual compounds as determined in the repeatability experiments. This is a conservative approach to determine discrimination between concentrations, both methods are likely to be more discriminative if larger numbers of replicates are analysed at different concentration levels. The cautiousness is due to the fact that presently repeatability was determined at one concentration level.

A more general aspect of discrimination between concentrations is that it not only depends on the sensitivity of the assessors, but also on the steepness of the concentration/detection frequency and concentration/intensity slopes. Steeper slopes will more quickly result in significant differences. For that reason 2-butanone, ethyl butyrate and hexanal having relatively steep slopes are more likely to show significant differences between concentrations.

Another way to compare the two methods is to cumulate the responses of all compounds per concentration (Fig. 3), i.e. to leave out the dimension of the individual compounds, as a sort of overall intensity for a particular sample. The detection frequency method showed lower coefficients of variance (3.7%) than the intensity method (11.7%). Both coefficients for the methods were lower for the cumulated response than for the individual compounds. Additionally, the cumulated response and the log concentration correlated more significantly than the individual compounds and the concentration. The correlation coefficient of the cumulated detection frequency and log concentration ($r = 0.99$) was higher than the one for the cumulated intensity ($r = 0.94$). However, the curve of the detection frequency levelled off slightly at the highest concentration level, whereas the intensities were still increasing log linearly with the concentration (Fig. 3).

4. Conclusions

The range of sensitivity for the detection frequency and intensity method was similar and covered the whole concentration range from sub-threshold to the maximal loading of the instrument. Robustness of the detection frequency method was shown in better repeatability. The intensity method resulted in higher discrimination between different concentration levels of the six volatile flavour compounds.

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