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Evaluation of three gas chromatography-olfactometry methods: comparison of odour intensity-concentration relationships of eight volatile compounds with sensory headspace data

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Abstract

Three gas chromatography–olfactometry methods, i.e. dilution analysis, detection frequency method and posterior intensity method, were evaluated for attribution of odour potency to eight volatile compounds at seven concentration levels. Six serial 1:5 dilutions of a solution consisting of 2-butanone, diacetyl, ethyl acetate, 3-methyl-1-butanol, ethyl butyrate, hexanal, 2-heptanone, α-pinene in pentane were analysed by gas chromatography-olfactometry. In addition, sensory odour intensity of sunflower oil solutions of the compounds at eight concentration levels were determined and related to their headspace concentrations. Posterior intensities correlated reasonably well with sensory odour intensities. Detection frequency data were also highly correlated, whereas dilution analysis led to more diverting results. A large variance among subjects was observed. Therefore, a group of assessors is considered a prerequisite for reliable gas chromatography-olfactometry analysis. © 2001 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Long lists of volatiles have been developed over the last decades (Maarse & Visscher, 1991). There are indications that only a small fraction of the large number of volatiles occurring in food actually contributes to the aroma or odour of foods (Guth & Grosch, 1999). Gas chromatography is an important analysis technique for aroma analysis. Although chemical detectors provide relevant information on volatile composition, many of them are not as sensitive for odour active compounds as the human nose (Acree & Barnard, 1994). Gas chromatography–olfactometry (GC–O) was proposed by Fuller and co-workers as early as 1964 and has shown to be a valuable method for the selection of odour active compounds from complex mixtures (Grosch, 1993). Initially, the GC effluent was sniffed and when an odour was perceived a description was given. GC–O is limited to this screening for odour active compounds, unless the chemical stimuli and the assessors' responses are quantified.

Several techniques have been developed over the last decades to collect and process GC–O data and to estimate the sensory contribution of single odour active compounds. Dilution analysis is a commonly used technique and was developed by two research groups: Grosch and co-workers developed the Aroma Extract Dilution Analysis (AEDA; Ullrich & Grosch, 1987) and Acree and co-workers CharmAnalysis (Acree, Barnard, & Cunningham, 1984). In dilution analysis an extract is diluted, and each dilution is sniffed until there are no longer any odours detected. In AEDA, the dilution factor (FD factor) is simply the last dilution at which an odour active compound is detected. In CharmAnalysis, the beginning and end of each odour are recorded. Duration of the individual detections are combined and graphed to yield a chromatogram with peaks and quantified peak areas (Charm values). Dilution analysis has been used to select odour active compounds and to determine their potency in a large number of food products, among which are bread, beef, coffee, herbs, oil, beer and mushrooms (Grosch, 1993).

The second method, the detection frequency method, was first proposed by Roozen and co-workers (Linssen, Janssens, Roozen, & Posthumus, 1993). It uses the number of assessors detecting an odour in the GC

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effluent simultaneously (detection frequency) as a measure for the intensity of a compound. Usually a group of 6–12 assessors assess a specific sample. The method has been used for determination of odour active compounds in various foods, such as vegetables, oils, emulsions, coffee, chocolate and spices (van Ruth, 2000).

A third group of GC–O techniques are posterior intensity methods, which measure the odour intensity of a compound in the GC effluent. The perceived intensity is scored on a scale after a peak has eluted from the GC column. The method has sporadically been reported in literature: it has been used to evaluate volatile compounds of fruits (Casimir & Whitfield, 1978) and dairy products (Arora, Cormier & Lee, 1995; Cadwallader & Howard, 1998).

The fourth group of methods involve time-intensity techniques, also called Osme. Assessors directly record the intensity with time for each compound detected. Only a few applications of the technique have been reported, e.g. for wine (Miranda-Lopez, Libbey, Watson, & McDaniel, 1992) and apples (Plotto, Mattheis, Lundahl, & McDaniel, 1998).

GC–O methods have often been applied. However, methodological aspects of GC–O have not received much attention. Only a few related studies have been reported and they were mainly focused on the influence of the extraction method on the composition of odour active compounds using real food systems (Abbott, Etiėvant, Langlois, Lesschaeve, & Issanchou, 1993; Moio, Chambellant, Lesschaeve, Issanchou, Schlich, & Etiėvant; van Ruth, Roozen, & Cozijnsen, 1995). Although composition of the aroma isolate is of great importance, the problem can be overcome by collection of volatiles from nose or mouth, or under mouth conditions. In that case the isolate is closely related to the quantities and proportions expressed when food is consumed.

Users of the various GC–O methods assume that they select all odour active compounds and rank them correctly in order of odour potency or intensity. However, as they are based on different principles and have not been compared together for one and the same sample, this is uncertain. A comparison of GC–O methods with sensory headspace analysis would allow further validation of the methods and reveal any effect of the temporal elution pattern at the GC sniff port.

The present study deals with the evaluation of three GC–O methods: posterior intensity method, detection frequency method and dilution analysis. The study is focused on attribution of odour potency or intensity to eight volatile compounds in a large concentration range by three commonly used GC–O methods. Perceived odour intensities in GC–O are compared with perceived intensities of the same compounds in sensory headspace analysis. Relationships between intensity/concentration functions of the compounds determined by GC–O and sensory analysis are examined.

2. Materials and methods

2.1. Materials

For instrumental analysis, a reference mixture of eight odour active compounds in pentane (Sigma-Aldrich, Steinheim, Germany) consisted of: 2-butanone (12.5 mg ml^{-1}; Sigma-Aldrich, Steinheim, Germany), diacetyl (2.5 mg ml^{-1}; Sigma-Aldrich, Steinheim, Germany), ethyl acetate (62.5 mg ml^{-1}; Sigma-Aldrich, Steinheim, Germany), 3-methyl-1-butanol (12.5 mg ml^{-1}; Lancaster, Walkerburn, UK), ethyl butyrate (2.5 mg ml^{-1}; Merck-Schuchard, Hohenbrunn, Germany), hexanal (12.5 mg ml^{-1}; Sigma-Aldrich, Steinheim, Germany), 2-heptanone (12.5 mg ml^{-1}; Sigma-Aldrich, Steinheim, Germany) and α-pinene (62.5 mg ml^{-1}; Sigma-Aldrich, Steinheim, Germany). Six serial dilutions (1:5) of the reference mixture were prepared by stepwise dilution with pentane.

For sensory analysis, individual solutions of the eight odour active compounds in sunflower oil (Mazola-pure sunflower oil; Bestfood UK Ltd, Esher Surrey, UK) were prepared: 2-butanone (16 mg ml^{-1}), diacetyl (0.005 mg ml^{-1}), ethyl acetate (16 mg ml^{-1}), 3-methyl-1-butanol (16 mg ml^{-1}), ethyl butyrate (0.005 mg ml^{-1}), hexanal (0.005 mg ml^{-1}), 2-heptanone (16 mg ml^{-1}) and α-pinene (16 mg ml^{-1}). Seven serial dilutions (1:5) of the individual solutions were prepared by stepwise dilution with sunflower oil.

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, UK). Cryogenic focusing was applied on the analytical column (SGE CTS.LCO2, Kiln Farm Milton Keynes, UK) 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, UK). 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 and dilutions (average coefficient of variance of replicates and between dilutions $<10\%$).

Eight assessors (women, aged 30–50 years) 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 earlier, which varied in concentrations. Assessors used laptop computers with a program in Pascal for data collection (Linssen et al., 1993). 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 nine-points intensity interval scale (1 = extremely weak, 9 = extremely strong) after their odour detection.

The reference mixture and its dilutions 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. Additionally, a blank sample with the solvent pentane was analysed. The panel average intensity scores were calculated for each dilution and the numbers of assessors detecting the compounds were determined (detection frequency). For dilution analysis, the dilution factor was determined by the last dilution at which the signal was above noise level.

2.3. Sensory analysis

An experienced panel of 10 judges (aged 30–50 years) was trained for analytical sensory analysis of the samples. Odour attributes were generated in focus groups (buttery, chemical, fruity, green leaves, pine, sweet). The solutions (5 ml) of eight odour active compounds in sunflower oil and a blank sunflower oil sample were served to the panel at room temperature in 20-ml vials. Overall odour intensity of the reference solution and its dilutions was evaluated by rating perceived intensities at a nine-points interval scale (1 = extremely weak, 9 = extremely strong).

2.4. Statistical evaluation

Panel average intensity scores were calculated for both GC–O and sensory analysis, intensity scores were subjected to Friedman two-factor ranked analysis of variance. Intensity/concentration slopes were calculated by log linear regression and Pearson's product-moment correlation coefficients (r) were determined. Spearman's ranked correlation coefficients were calculated to compare the GC–O data obtained by the three different methods and to compare GC–O and sensory data (O'Mahony, 1986). A significance level of 5% was used throughout the study.

3. Results and discussion

The three GC–O methods, posterior intensity method, detection frequency method and dilution analysis were compared for determination of odour intensity/potency of a mixture of eight volatile compounds in a wide concentration range, i.e. 2-butanone, diacetyl, ethyl acetate, 3-methylbutanol, ethyl butyrate, hexanal, 2-heptanone and α-pinene. The components are all known to contribute to the aroma of foods (Arctander, 1994). When a dummy sample was evaluated maximal two assessors perceived an odour simultaneously. The same noise level was obtained when the solvent pentane was analysed. Therefore, a response by three assessors or more is considered a signal.

3.1. Posterior intensity method

The results of the posterior intensity methods, i.e. average intensity scores of the eight volatile compounds for the reference mixture and its six dilutions, are presented in Table 1. Large differences in odour intensity

Table 1
Average intensities and detection frequencies (in brackets) of eight odour active compounds in a reference mixture and in six dilutions determined by gas chromatography olfactometry ($n = 8$)

Compound	Reference	Dilutions					
		1:5	1:25	1:125	1:625	1:3125	1:15,625
2-Butanone	6.1 (8)	4.0 (7)	–[a]	–	–	–	–
Diacetyl	6.8 (8)	4.9 (7)	4.1 (7)	3.5 (7)	1.5 (3)	1.0 (3)	–
Ethyl acetate	3.1 (4)	1.6 (3)	–	–	–	–	–
3-Methyl-1-butanol	5.0 (7)	4.3 (6)	–	–	–	–	–
Ethyl butyrate	6.8 (8)	5.4 (7)	3.5 (5)	–	–	–	–
Hexanal	6.6 (8)	4.6 (8)	2.5 (6)	1.8 (4)	–	–	–
2-Heptanone	4.3 (6)	–	–	–	–	–	–
α-Pinene	1.3 (4)	1.1 (3)	1.1 (3)	–	–	–	–

[a] At or below noise level (detection by two or one assessors).

between the odour active compounds were observed. 2-Heptanone could only be detected in the reference mixture, whereas 2-butanone, ethyl acetate and 3-methyl-1-butanol could also be detected in the first dilution. Diacetyl was detected in most dilutions. In the reference mixture, 2-butanone, diacetyl, ethyl butyrate and hexanal showed highest odour intensity scores, which did not differ significantly (Friedman two factor ranked analysis of variance, $P<0.05$). α-Pinene had lowest intensity, despite its high physical concentration in the reference mixture (62.5 mg ml^{-1}). Large quantities of volatile compounds do not necessarily relate to high odour intensities, as this property is due to differences in thresholds and differences in intensity/concentration relationships.

The compounds diacetyl, ethyl butyrate, hexanal and α-pinene could be detected in a sufficient number of dilutions to calculate intensity/concentration slopes (psychophysical functions). Table 2 lists d(intensity)/d(concentration) for the mathematical relationships of the four compounds. The log linear relationship (intensity-ln concentration) showed significantly higher correlation coefficients than power and linear functions (Pearson's product-moment correlation coefficient, $P<0.05$). The log linear relationship is in agreement with Fechner's law. This law states that the sensory intensity of a compound is log linearly related to its physical concentration (Sauvageot, 1990). The intensity/concentration slopes were different for the four compounds. α-Pinene had a very low slope value, whereas higher values were obtained for diacetyl. Ethyl butyrate and hexanal had similar, as well as the highest slope values of the four compounds. Although the specific compounds have not been studied before, results agree with other studies, which showed different slopes for aroma compounds in sensory analysis (Patte, Etcheto, & Laffort, 1975).

3.2. Detection frequency method

The detection frequencies for the eight compounds in the reference mixture and the dilutions were determined (Table 1). In the reference mixture 2-butanone, diacetyl, ethyl butyrate and hexanal showed the maximum detection frequency, which is eight assessors. Ethyl acetate and α-pinene could be detected in the reference mixture by only four assessors. A concern of the detection frequency method is that its use is limited to the concentration at which the maximum frequency possible is attained (= number of assessors). In this particular case, hexanal is the only compound detected by all assessors in one other sample but the most concentrated one. All the other compounds did not reach the maximum frequency or reached it in the reference mixture only. It should be kept in mind, that the concentrations were rather high in the reference mixture, i.e. the most concentrated sample. For this sample, concentrations at the sniff port varied from 100 ng/compound for diacetyl and ethyl butyrate, to 2500 ng for α-pinene. The concentration range covered the amounts usually analysed in aroma analysis (0–100, 0–500 or 0–2500 ng). It is remarkable that for most of those concentrations, there is always a part of the assessors not able to detect these compounds.

Detection frequencies decreased with further dilution for each of the compounds. The intensity/ln concentration relationship of four compounds is presented in Table 2. The correlation coefficients show a reasonable correlation between the log transformed concentration and the detection frequency. These results are in agreement with previous studies, which showed a linear relationship between the amount of 2- and 3-methybutanal, and hexanal released from rehydrated French beans and the number of assessors detecting compounds (van Ruth, Roozen, & Cozijnsen, 1996). Diacetyl and α-pinene had lower slope values, which indicates that thresholds of the assessors are spread over a wider concentration range for these compounds than for the others. This might be due to various causes, e.g. anatomy and psychological factors might play a role. The ranking of the slope values [d(detection frequency)/d(ln concentration)] was identical to those of the odour intensity scores. High correlation coefficients were obtained between the average intensity scores and the detection frequencies of the individual compounds at all concentration levels (Spearman's ranked correlation; $r=0.920$).

3.3. Dilution analysis

The odour potencies of the eight volatile compounds in the reference mixture determined by dilution analysis are presented in a dilution aromagram in Fig. 1, which shows the dilution factor vs. retention indices. Using this method, the ranking order of the compounds for odour potency from high to low is: diacetyl, hexanal, ethyl butyrate, α-pinene; 2-butanone, ethyl acetate, 3-methylbutanol; and 2-heptanone. The dilution values

Table 2
Four odour active compounds of the reference mixture and their change in intensity with relative concentration: slopes and Pearson's correlation coefficients (r) for odour intensity (average odour intensity/ln concentration) and for detection frequency (detection frequency/ln concentration)

Compound	Intensity		Detection frequency	
	Slope	r	Slope	r
Diacetyl	0.707	0.969	0.657	0.788
Ethyl butyrate	1.3297	0.965	1.243	0.952
Hexanal	0.944	0.970	0.994	0.941
α-Pinene	0.186	0.763	0.373	0.900

for the compounds in the reference mixture (ln dilution factor) correlated slightly with odour intensity scores and detection frequencies of the reference mixture (Spearman's ranked correlation; $r=0.753$ and $r=0.661$, respectively). Relatively large dilution steps were used (1:5) in order to cover the concentration range from maximum loading of the GC-column to threshold (15,625:1). However, smaller dilution steps might have improved correlations between dilution step and odour intensity. Presented dilution analysis data differed more from the posterior intensity data, than the detection frequency results before. Dilution analysis considers intensity/concentration slopes of odour active compounds to be similar. Nevertheless, as was shown in Table 2, the slopes of the compounds differed considerably.

Dilution factors for individual compounds varied over a range of four dilutions ($5^4\times$lowest concentration) for the assessors, demonstrating the large variability in threshold. This is due to differences in thresholds and response criteria of assessors (Kleykers & Schifferstein, 1995). Odour thresholds generally show a large variation, i.e. up to a factor 200 (Meilgaard, 1993), which can be attributed to differences in age and type of the assessors, flow rate of the stimuli, and experience with the experimental procedure (Punter, 1983). Procedures, which base determination of the dilution value or even selection of odour active compounds on assessment of one or two assessors should, therefore, be called into question. Regarding the present results, authors consider a group of assessors a prerequisite for reliable GC–O analysis.

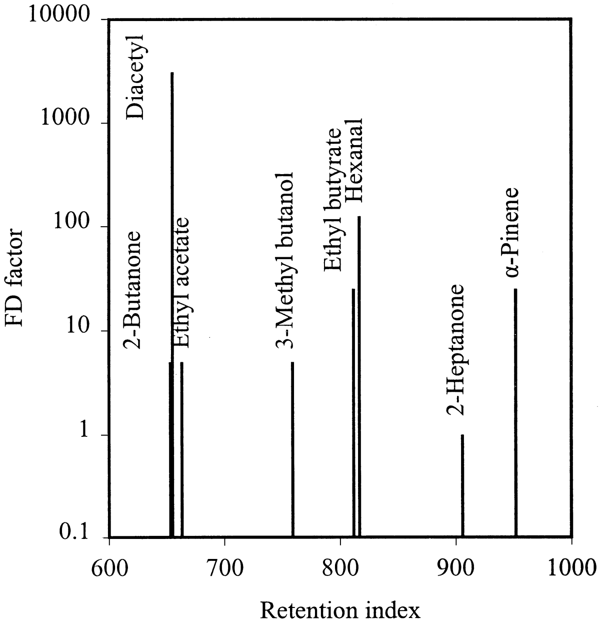

Fig. 1. Dilution aromagram of eight odour active compounds in pentane (reference mixture). FD = dilution factor.

3.4. Sensory odour analysis

The sensory odour intensity of the eight compounds dissolved in sunflower oil was determined in order to relate them to the intensity determined in GC–O analysis. The odour intensity of individual solutions of the compounds, seven serial dilutions and a blank sunflower oil sample was assessed (Table 3). The concentrations in the headspace of the samples were estimated by conversion of liquid concentrations to headspace concentrations by gas/sunflower oil partition coefficients published by the authors elsewhere (van Ruth, Grossmann, Geary, & Delahunty, 2000). Again, intensity/concentration slopes varied considerably among the compounds. The last concentration of the samples above noise level (= intensity blank sample) related reasonably well to the last concentration above noise level in GC–O (Spearman's ranked correlation; $r=0.881$). The similarity between the intensity/concentration functions in GC–O and in sensory odour analysis is graphically presented for three of the compounds in Fig. 2. The curves of the three compounds showed similarity, although α-pinene was less similar than those of the other compounds. This might be due to presence of small impurities in the samples, which did

Table 3
Average odour intensities of eight odour active compounds in sunflower oil and average coefficients of variance: blank sunflower oil sample, reference mixture (ref) and six dilutions (1:5; 1:25; 1:125; 1:625; 1:3125; 1:15,625) determined in sensory analysis ($n=8$)

Compound	Blank	Ref	1:5	1:25	1:125	1:625	1:3125	1:15,625	1:78,125
2-Butanone[a]	1.1	8.3	6.0	4.1	1.2	1.1	1.2	1.6	1.3
Diacetyl[b]	1.6	6.0	5.4	4.5	2.1	1.2	1.7	1.4	1.3
Ethyl acetate[a]	1.2	7.9	6.7	4.3	1.7	1.6	1.3	2.3	1.4
3-Methyl-1-butanol[a]	1.4	6.9	6.1	5.7	2.7	1.6	1.7	3.2	1.7
Ethyl butyrate[b]	1.5	5.0	5.3	3.6	1.7	2.0	1.4	1.2	1.4
Hexanal[b]	1.4	3.0	3.2	3.3	1.7	1.6	2.0	2.0	2.0
2-Heptanone[a]	1.3	7.1	6.5	3.7	5.6	2.7	1.3	1.5	1.6
α-Pinene[a]	1.2	7.7	5.5	3.6	2.1	2.9	1.2	1.2	2.3
CV [%]	40	28	35	42	45	54	45	56	49

[a] Concentration of compound in individual solution: 16 g l^{-1}.

[b] Concentration of compound in individual solution: 0.005 g l^{-1}.

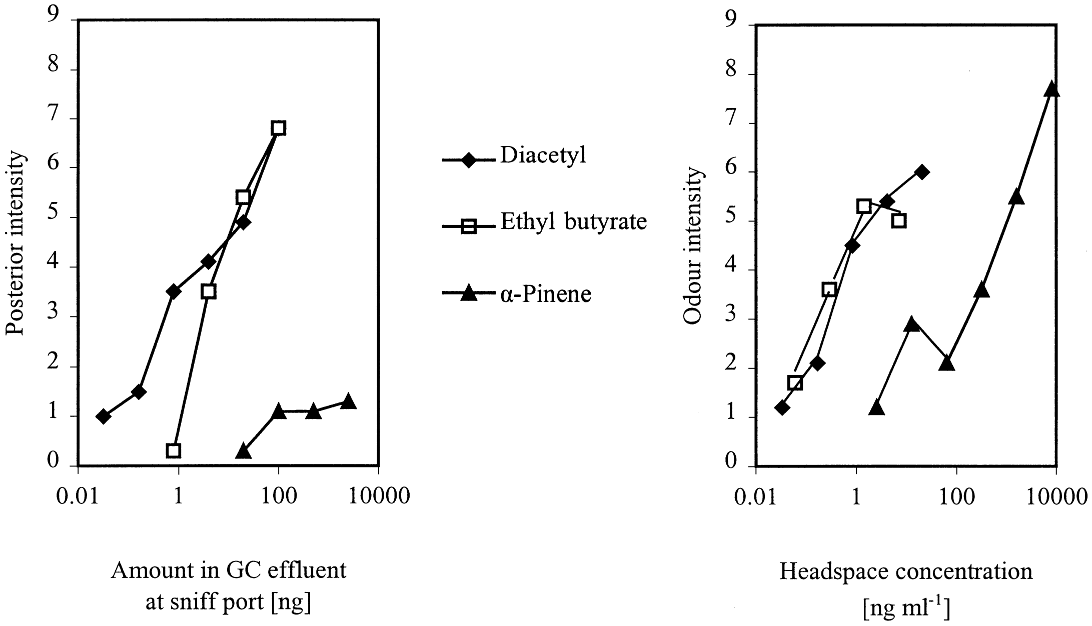

Fig. 2. Relationships between intensity scores and concentrations for diacetyl, ethyl butyrate and α-pinene determined by (A) gas chromatography-olfactometry analysis and by (B) sensory headspace analysis.

not affect GC–O data, but did affect sensory data. Terpenes are usually obtained by essential oils and can therefore be contaminated by trace components. GC–O data and sensory data were further quantitatively compared. GC–O posterior intensity scores for the eight compounds and sensory odour intensity scores related reasonably well for similar quantities of compounds (Spearman's ranked correlation; $r = 0.821$, concentration level: 100 ng per compound at each sniff port for GC–O and 100 ng/ml in sensory analysis).

The correlation between GC–O posterior intensity scores and sensory odour intensity scores confirms the correct attribution of odour intensity of single compounds by this GC–O method. It is remarkable that the temporal pattern of the compounds eluting from the analytical column in GC–O did not affect its similarity with sensory headspace data. The high correlation between GC–O posterior intensity scores and detection frequencies also shows potential for the latter method, which involves a more simplified task for the assessors and requires less training. Unfortunately, dilution analysis, which is based at a different principle, leads to more diverting results.

4. Conclusions

In the present study, GC–O data obtained by posterior intensity and detection frequency methods resulted in very similar odour intensity/concentration relationships as sensory headspace analysis. Odour potency attribution by dilution analysis demonstrated more diverting data. Large differences in thresholds and individual intensity/concentration curves among assessors leads to the conclusion that a larger group of assessors is required for reliable GC–O analysis independent of the method used.

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