Effects of the Sniffing Port Air Makeup in Gas Chromatography−Olfactometry†

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A time−intensity gas chromatography−olfactometry (GCO) apparatus was developed to study some aerodynamic parameters that may influence odor detection and intensity measurements by the subjects. The addition of humidified air at the elution place of the compounds is generally recommended for several reasons (essentially to prevent nasal mucosa dehydration and to improve chromatographic effluent carriage out of the column), but clues about these effects are yet to be published. This question is studied through two complementary experiments using synthetic solutions of 3-methyl-1-butanethiol, hexan-2-one, octanal, nonanal, furfural, citronellal, benzaldehyde, octan-1-ol, 2-phenylethyl acetate, guaiacol, 2-phenylethanol, and vanillin. This work demonstrates the need for an air makeup to increase odor detection frequency and intensity rating. With the conditions tested, a minimum makeup air flow rate of 50 L·min^{-1} is necessary. On the contrary, humidification of the makeup is useless for the sniffers comfort and to improve the quantitative GCO results.

Keywords: *Gas chromatography−olfactometry; odor intensity; air makeup; sniffing port*

INTRODUCTION

Gas chromatography−olfactometry (GCO) provides a sensory profile of the odor active components present in an aroma extract by sniffing the GC effluent. Although GCO does not take into account the sensory interactions arising when eating food (between odorants, and between odorants and nonodorants, Piggott, 1990), this technique has proved to be useful for determining key odorants in food products, either for food aroma composition understanding, flavor creation, or off-flavor identification (Guichard, 1992, Mistry et al., 1997, Blank, 1997). The common GCO methods essentially differ from one another in the technique used to determine the individual odorous potency of the compounds. Rationalization of GCO was initiated by Acree et al. (1984) with CHARM, and by Ullrich and Grosch (1987) with AEDA, two techniques based on extract dilution. In these methods, the contribution of each odor is worked out by a value calculated with the odor detection threshold. Miranda-Lopez et al. (1992) and Etiévant et al. (1999) developed two different time−intensity methods respectively called OSME and GC-O-FSCM: each odor intensity is directly evaluated by trained subjects. Finally, Pollien et al. (1997) proposed a method based on the frequency of odor detection determined by an untrained panel. The dilution techniques have been largely criticized as based on a concept of odor unit which is inconsistent with the psychophysic laws (Abbott et al., 1993). Furthermore, they are very time-consuming methods because of the successive dilution evaluations needed until no more odor is detected. Thus, we chose to use an Osme-like method using a PC mouse device to score the intensity, as already tested by Guichard et al. (1995). Our purpose is to study some aerodynamic and physiological factors that can contribute to the variability of the GCO responses. We focused our study on the coupling between the gas chromatograph and the subject who sniffs. The addition of humidified air at the sniffing outlet has been generally applied since the first improvements of GCO methods (Dravnieks and O'Donnell, 1971). It is said to prevent nasal mucosa dehydration, to avoid condensation of the stimulus on the walls of the sniffing port (Drawert and Christoph, 1984), and to preserve the resolution of the narrow bore columns which can have less than 1 mL·min^{-1} carrier gas flow rate (Acree, 1993). Various air flow rates are described: from 11 mL·min^{-1} by Miranda-Lopez et al. (1992) to 100 mL·min^{-1} by Drawert and Christoph (1984), but no experiment is cited which demonstrates the usefulness of air addition or humidification, and the choice of the flow rate is not explained either. Thus, the aims of this paper are to experimentally check the need for an air makeup (first experiment), to study the effect of the air flow rate (second experiment), and to see if humidification, which is sometimes difficult to maintain without producing drop expulsion, is actually justified (second experiment).

MATERIALS AND METHODS

Synthetic Solution. The compounds were essentially purchased from Aldrich (Saint-Quentin Fallavier, France), or generously provided from René Laurent S.A. (Le Cannet, France). Their choice was influenced by Etiévant et al., (1999) who used them mainly because they have known Stevens' exponents (Devos et al., 1998), different chemical functional-

† This work was partly presented at the IIIrd Pangborn Sensory Science Symposium, August 1998, in Alesund, Norway.

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10.1021/jf990913g CCC: $19.00
Published on Web 04/29/2000

Table 1. Composition of the Synthetic Solution Used in the First Experiment

compound	concentration ($g \cdot L^{-1}$)
3-methyl-1-butanethiol	0.16
benzaldehyde	14.48
guaiacol	4.60
2-phenyl-1-ethanol	10.57
vanillin	5.98

Table 2. Composition of the Synthetic Solutions Used in the Second Experiment

compound (classified in elution order)	concn ($g \cdot L^{-1}$)	solution number x = occurring in the solution 1	2	3	4	5	6
3-methyl-1-butanethiol	0.11	x	x		x	x	x
hexan-2-one	15.01	x		x	x	x	x
octanal	5.01	x	x	x	x	x	
nonanal	1.03		x	x	x	x	x
furfural	15.06	x	x		x	x	x
citronellal	5.01	x	x	x	x	x	
benzaldehyde	10.02	x	x	x	x		x
octan-1-ol	10	x	x	x		x	x
2-phenylethyl acetate	5	x		x	x	x	x
guaiacol	0.51	x	x		x	x	x

ities, and volatilities. For the present experiment, we were also interested by the chemical variety of these compounds occurring in food products. Moreover, they could be eluted in a time compatible with short sniffing experiments. In the first experiment, five compounds were chosen in order to be eluted without coelution within 15 min. Their relative concentrations in ethanol (Table 1) were adjusted in order to produce weak and intense odorous stimulations for the panelists during an analysis. In the second experiment, eight compounds from Etiévant's list plus octanal and phenylethyl acetate were chosen. They were diluted in dichloromethane and distributed in six solutions. Each compound had the same concentration in each solution, but was present in five of the six solutions in order to avoid memory effects (Table 2).

Chromatographic Conditions. Both experiments were performed on a Hewlet-Packard 5890 gas chromatograph equipped with the HP 5895A GC Chemstation software on a Pascal Station (Hewlett-Packard, Les Ulis, France). One microliter of each solution was injected in a split-splitless injector (250 °C; split ratio = 1:7.3 in the first experiment, 1:2.9 in the second). The compounds were separated into two different columns depending on the experiment: HP-5 in the first (Hewlet-Packard; 24 m × 0.32 mm; 0.52 μm thickness) and DB-WAX in the second (J&W Scientific Inc.; 30 m × 0.32 mm; 0.5 μm thickness). The carrier gas was helium, at 1.2 $mL \cdot min^{-1}$ in the first experiment and at 3.8 $mL \cdot min^{-1}$ in the second. At the end of the column, helium flow was split into two equal parts, one going to the flame ionization detector (FID, at 250 °C), and the other going to the sniffing port. The split occurred through an SGE capillary splitter (SGE, Villeneuve Saint-Georges, France) connected to two fused silica deactivated capillary tubing of the same length. Each experiment was performed with a different oven temperature program: 4 $°C \cdot min^{-1}$ from 120 °C to 180 °C, and 20 min at 180 °C in the first experiment; 6 $°C \cdot min^{-1}$ from 70 °C to 140 °C, and then 30 $°C \cdot min^{-1}$ from 140 °C to 200 °C, and 7 min at 200 °C to elute the 10 compounds in less than 20 min in the second experiment.

Olfactometric Conditions. The capillary connection to the sniffing port was left at a warm temperature through two types of transfer lines. In the first experiment, we used a 7 cm long SGE transfer line (SGE Olfactory Detector ODO-1) situated on the top of the oven and heated by a venturi tube. A glass cone sniffing port was placed at its top end. To test the influence of an air makeup, an unhumidified air flow was added concentrically to the chromatographic effluent at the bottom of the glass cone. The condition without air was compared to three conditions with air added at 25, 200, or 500 $mL \cdot min^{-1}$. In the second experiment, a transfer line made up from a flexible 60 cm long tube heated by a resistance at 200 °C was used. This line ensured a more comfortable position for the sniffers and probably avoided volatile compound condensation during their transfer to the sniffing port. Actually, we observed that after the elution of vanillin, its residual odor disappeared far more rapidly with the electrically heated transfer line than with the venturi transfer line. As in the first experiment, air was added concentrically at the glass cone bottom fixed at the end of the transfer line. Different flow rates were tested: 50, 100, and 200 $mL \cdot min^{-1}$, humidified or not. Humidification was obtained by bubbling air in distilled water. Sniffers were never informed of the different conditions involved. In both experiments, sniffers were invited to tell about any discomfort during the analyses. At the end of each experiment they were directly asked if they had noticed nasal dryness.

Panel and Sensory Data Acquisition. Two men and two women, aged 22 to 29 years old, formed the panel of the first experiment. Only one subject had already experimented sniffing, one year before. They were trained during two sessions in order to get familiar with the apparatus. For the data acquisition, the four conditions of air flow rate were repeated three times for each sniffer and presented in a random sequence. The subjects had to indicate the beginning, the end, and the maximum odor intensity of each odor (I_{max}) by a click with a PC mouse on a computer user interface developed at ENSIA. The intensity was noted on a 10 cm long unstructured linear scale displayed on the computer screen in front of the subject. Subjects were also asked to describe the odors detected.

The second panel was made up of six women, aged 23 to 42, who had not taken part in any sniffing experiment before. Familiarization was more gradual, beginning with one stimulation at one intensity and finishing with the 10 odorous compounds at different intensities (procedure not detailed). The same software was used but in a simpler way: subjects had to click once with the PC mouse at the extreme left of the scale (zero) to indicate the sensation start, and to click a second time on the scale at the end of the sensation to match the intensity. Injections of the six solutions allow each compound to be detected five times (Table 2). As there were six different air makeups (three air flow rates × two hygrometric conditions), the subjects performed 36 analyses in order to have five replicates of the same air makeup conditions for each compound (in a random sequence).

Stastistical Analysis. Analyses of variance were performed with STAT-ITCF (ITCF, Paris, France) in the first experiment, and with the SAS General Linear Model procedure (SAS 6.12; SAS Institute Inc., Cary, NC) in the second experiment.

RESULTS

For the first experiment, a two-class variance analysis (judges and air flow rates) with interactions was performed component per component on the I_{max} values. Besides the judge effect, significant at the 5% level except for the component 3-methyl-1-butanethiol, the air flow rate has a significant effect for each component at 1% level. A Newman−Keuls test on the means shows significant differences with and without air at the 5% level. Each time the intensity means are higher with 200 and 500 $mL \cdot min^{-1}$ air flow. Figure 1 shows that the panel frequency of detection is better at the two highest air flow rates, and the mean intensities calculated without the null values (Figure 2) are also higher with air added. These results indicate that an addition of air at the sniffing outlet increases the frequency of detection and the perceived intensity of the odors.

At the same time as these observations, the response delays (sensation start time − FID retention time) for two of the sniffers decrease with higher air flow rates. It is particularly true with one, whose delays vary

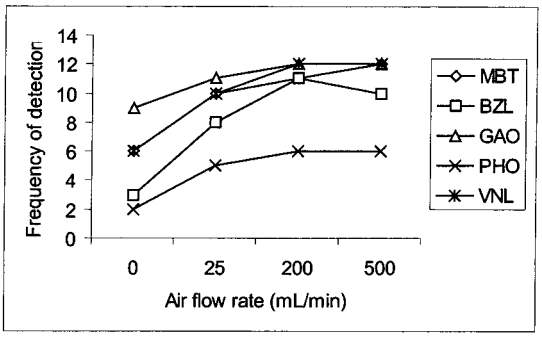

Figure 1. Effect of makeup flow rate on panel frequency of detection. MBT: 3-methyl-1-butanethiol; BZL: benzaldehyde; GAO: guaiacol; PHO: 2- phenyl-1-ethanol; VNL: vanillin.

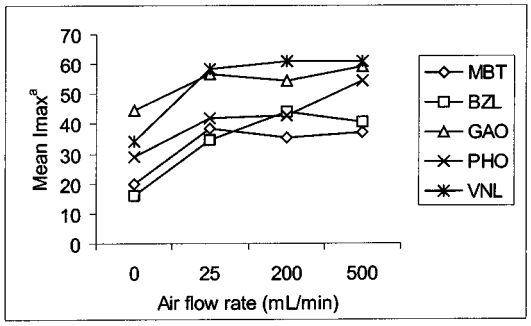

Figure 2. Effect of makeup flow rate on mean Imax of the panel. MBT: 3-methyl-1-butanethiol; BZL: benzaldehyde; GAO: guaiacol; PHO: 2-phenyl-1-ethanol; VNL: vanillin. [a]- The averages are calculated without the null values.

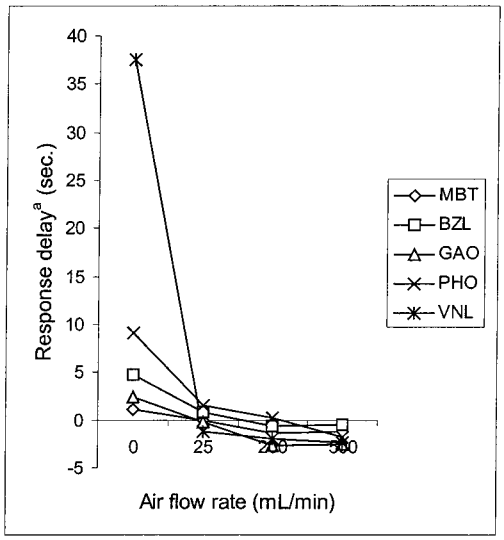

Figure 3. Effect of makeup flow rate on subject response delay (first experiment). MBT: 3-methyl-1-butanethiol; BZL: benzaldehyde; GAO: guaiacol; PHO: 2-phenyl-1-ethanol; VNL: vanillin. [a]Response delay = sensation start time − FID retention time.

between −2.7 and 9.15 s, and even 37.5 s for vanillin without air (Figure 3).

Concerning air humidification, none of the four sniffers complained about nose dryness when asked, although the additional air was not humidified.

As most differences were observed between the condition "no air added" and "air added", the second experiment tested the influence of intermediate air flow rates (50, 100, or 200 mL·min^{-1}) and air humidification. Data about furfural were dropped because they were rare and unconfirmed by the odor description. This compound was probably eluted under its concentration threshold. The SAS General Linear Model procedure (GLM procedure: SAS analysis of variance with fixed classes) was applied on the intensity data, component per component, with the following classes: subjects (six levels), replicates (five levels), air flow rates (three levels), hygrometry (two levels), and the six possible two by two interactions. Neither hygrometry nor flow rate show significant effects although the combination "200 mL·min^{-1} and humidified air" is significantly higher for 3-methyl-1-butanethiol ($p = 0.002$), and the combination "50 mL·min^{-1} and unhumidified air" is significantly higher for nonanal ($p = 0.012$). The application of a mixed analysis of variance (subjects and replicates classes randomized, hygrometry and flow rates fixed) did not give more significant results. So, the second experiment exhibited no global effect of air humidification or air flow rates changes between 50 and 200 mL·min^{-1} on the intensity measurement. However, as the first analysis of variance model explained only 47% (R^2 for guaiacol) to 66% (R^2 for nonanal) of the variance, two other possible factors were investigated. The first one was the sequence order of the 36 sessions achieved by each subject, but the GLM procedure showed no significant session order effect. The second one was a cross-adaptation between two components consecutively eluted. As a matter of fact, Ekman et al. (1967) claimed that several minutes may be needed to recover one's initial sensitivity after an adaptation and in our experiment, the time between the retention times of two consecutive compounds varied from an average of 17 s to 207 s, and the minimum delay (start time of a compound − end time of the preceding compound) varied from 5 to 194 s. A Student test was carried out for each compound (Table 2) to compare its intensity values when its preceding compound was present and not, whatever the air flow rate or hygrometry applied, for these effects were not significant as seen above. Contradictory results were found: intensities for some of the compounds were significantly higher when the preceding compound was absent from the solution (as expected in cross-adaptation), whereas the contrary was observed for others. Furthermore, each case was due to only one sniffer, except for nonanal, which was more intensively noted by two subjects when octanal was absent, and more intensively noted by one subject when octanal was present. These contradictory results can be attributed to chance.

DISCUSSION

Air Flow Rates. When studying turtles' ability to perceive odors, Tucker (1963) observed that an odor is perceived if a sufficient number of molecules strike the olfactory mucosa within a critical time period. Schneider et al. (1966) confirmed this concept on humans, and according to Bowers and MacLeod (1972), this period of temporal summation would last 200 ms. This phenomenon could explain why the addition of air in the first experiment increased the detection frequency and the intensity of odors. We can suppose that without air the volatile molecules eluted were dispersed in the sniffing port and only a fraction of them reached the olfactory mucosa during the period of summation, and when air was added, all the molecules available at the capillary outlet were inspired by the sniffer and reached his neuroepithelium in this short period of time. Thus,

when air is added, the additional molecules can allow detection if conditions are near the detection threshold and can enhance the intensity in case of a suprathreshold condition. In the second experiment, 50 mL·min^{-1} was probably sufficient to obtain the best carriage, and a higher flow rate could not increase the perceived intensity because the restricting factor was the GC effluent flow rate and the concentration of molecules in the solution injected. Nevertheless, highly volatile chemicals were not tested in our experiments. It would be interesting to study the influence of lower and higher air flow rates than 50 mL·min^{-1} on the evaluation of low-boiling compounds such as acetaldehyde or ethyl acetate. In this case, headspace techniques would be more adapted, otherwise these compounds would be coeluted with the solvent.

Air Humidification. It is usually said that an addition of humidified air is needed to prevent nasal dehydration due to dry hot effluent at the sniffing port (Acree et al., 1984; Mistry et al., 1997). However, in our experiments, the sessions with or without humidification showed no difference, neither on the sniffers comfort nor on the GCO results. This lack of humidification effect could be predicted in our experimental conditions by comparing the GC carrier gas flow at the sniffing outlet in the first experiment (0.6 mL·min^{-1} of helium) and the additional air flow in both experiments (25 to 500 mL·min^{-1}) with the average flow rates of human normal breathing: 15 L·min^{-1} per nostril according to Willemot et al. (1971). Helium alone was diluted 50000 times in the air inspired by each nostril, and when air was added, the dilution factors varied from 60 to 1200. Indeed, such dilution factors may have little effect in the nose which is an efficient natural humidificator of the inspired air: relative humidity is near 95% in the nasopharynx (Willemot et al., 1971). Moreover, most of the subjects actually "sniffed" when they detected an odor, which corresponds to a 100% increase in the inspiratory flow rate (Laing, 1983), so the dilution is also doubled and the modification of the overall inspired air humidity is then not to be considered.

In conclusion, our experiments demonstrate the effective need of an air makeup to improve the molecules carriage efficiency out of the column. In our conditions, a minimum makeup air flow rate of 50 mL·min^{-1} is necessary, but its humidification is useless.

ACKNOWLEDGMENT

Thanks are particularly expressed to Dr. P. Etiévant (INRA Dijon), Dr. N. Vallet and M. Rogeaux from TEPRAL (Danone beverage research center, Strasbourg, France) for useful discussions and to TEPRAL for financial support.

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Received for review August 17, 1999. Revised manuscript received February 15, 2000. Accepted February 23, 2000.

JF990913G