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Amplification of Gas Chromatographic−Olfactometric Signal by Ethanol

JÁN PEŤKA,*,† ANA ESCUDERO,‡ JANA SÁDECKÁ,§ KRISTÍNA KUKUROVÁ,§ AND VICENTE FERREIRA‡

Akras Flavours, Industriezentrum NÖ Süd, Postfach 56, 2355 Wiener Neudorf, Austria; Department of Analytical Chemistry, Faculty of Sciences, University of Zaragoza, c/Pedro Cerbuna 12, 50009 Zaragoza, Spain; and Food Research Institute, Priemyselná 4, 824 75 Bratislava, Slovakia

An indication of the amplifying effect of ethanol vapors from a humidifier on the perceived odor intensity of eluting odorants was discovered in previous GC−olfactometric (GC-O) experiments. In this study were tested 12 volatiles belonging to various chemical classes to confirm this phenomenon. Two methods were used: the normal GC-O design, whereby panelists smell the effluent incessantly during the whole run, and a design applicable in a target-oriented quantitative GC-O, whereby panelists start sniffing just a short time before the olfactory event starts. Two hydroalcoholic solutions and pure water were put into the humidifier and tested in both designs. Continuous aspiration of ethanol vapors caused a decrease of average intensity for most of the selected volatiles. In contrast, a significant amplifying effect of ethanol on panel intensity was observed in the target-oriented design for most of the compounds. This observation was confirmed with an independent panel. The proposed modification of a standard GC-O procedure could remarkably enhance the performance of GC-O studies oriented on a few target chemicals.

KEYWORDS: GC-O; performance; increase

INTRODUCTION

The human nose has been used as a gas chromatography (GC) detector almost since the introduction of the technique itself (*1*), as it helps the analyst to pick out only those components of an extract that contribute significantly to the sensory sensation people have from the extracted material. The output of GC−olfactometry (GC-O) is quite often different from the output of the most common GC detectors (FID, MSD) due to the very different selectivity of the human nose. Because of this GC-O reveals trace chemicals present in the sample at concentrations well below the detection limits of FID or MSD. GC-O has since then developed into the most common analytical method employed in the process of screening for odor-active compounds in foods or other goods, in which the sense of smell can influence purchasing decisions.

Two types of GC-O methods are basically used: those that determine odor thresholds of the odor-active volatiles in air by dilution of the original aroma extract (Charm, AEDA) and those that estimate the intensity of the eluting odor-active volatiles (finger-span/OSME, posterior estimation of intensity, frequency methods) (*2*). Notwithstanding the fact that GC-O tracks single chemicals only, it can provide enough information for a successful reconstitution of the original complex smell (*3*). There are attempts to explore the quantitative potential of the human nose, too, either in comparative studies (*4*) or by direct quantification of volatiles (*5*, *6*).

In a previous study we investigated correlations between concentration−intensity trends of single flavor compounds dissolved in a wine-like medium (10% alcohol, pH 3.4) and the same kind of trends obtained from GC-O (*7*). The majority of GC-O dose−response functions were located over the corresponding functions obtained from tastings of the same compounds in hydroalcoholic solution, as expected due to complete volatilization and absence of obstacles (matrix, acidity, etc.). Opposite location of the decanal and eugenol dose−response functions raised questions, which we tried to explain, by other means, by placing a 10% hydroalcoholic solution into the humidifier of the sniffing port (*7*). New measurements focused on these two compounds showed that ethanol increased their odor intensity in the whole concentration range studied, that is, at the perithreshold level, too. This means, obviously, that using hydroalcoholic solutions in the humidifier instead of water could enhance the sensitivity of the sense of smell. Accordingly, it could make both qualitative and quantitative measurements of odors more precise and reliable.

In the current study we extended the previous experimental design with additional hydroalcoholic solution and tested a higher number of volatile organic compounds as well. Furthermore, we tested a hypothesis of the general amplifying effect

* Corresponding author (e-mail petkajan@unizar.es; telephone +43 2236 6255 0234; fax +43 2236 62443).
† Akras Flavours.
‡ University of Zaragoza.
§ Food Research Institute.

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Table 1. Concentrations in the Injected Solution (*c*), Linear Retention Indices (RI) on a DB-Wax Column, and the Most Common Odor Descriptions of the Selected Volatiles Generated by the Panels

compound	*c* (ppm)	RI	descriptor
ethyl butanoate	0.01	1040	fruity
cis-3-hexenal	0.05	1141	green, grassy
2-heptanone	9	1186	soapy
3-methyl-1-butanol	0.6	1219	fusel, yeasty
decanal	1	1502	orange
linalool	0.1	1560	floral
acetylpyrazine	0.08	1626	mousy
2-methylbutanoic acid	4	1681	cheese, sweat
methionol	1	1723	potato
α-ionone	1	1852	violet
γ-octalactone	0.08	1915	coconut
eugenol	0.2	2174	clove

of ethanol for all GC-O analyses, that is, for those performed during the whole GC run time uninterruptedly, too.

MATERIALS AND METHODS

Chemicals. The list of used chemicals, together with their concentrations, linear retention indices (*8*), and generated descriptors is showed in **Table 1**. The experiment was thoroughly designed to inspect various groups of volatile compounds and, at the same time, to ensure sufficient gaps between odors, to eliminate the possibility of cross-adaptation.

All chemicals were gifts from Bedoukian Research Inc. (Danbury, CT) except for dichloromethane, which was purchased from Merck (Darmstadt, Germany). Dichloromethane was twice rectified before use. Both panels sniffed the same mixture, physically, to ensure the same conditions.

Pure water was obtained from a Milli-Q purification system (Millipore, Bedford, MA). Ethanol absolute was from Panreac (Barcelona, Spain) and from Merck (Darmstadt, Germany), respectively.

GC. *Panel 1:* Sniffings were carried out at constant pressure (linear velocity of 34.5 cm s^{-1}, measured at 143 °C) on a Thermo Quest Trace (Rodano, Italy) gas chromatograph equipped with a FID and a sniffing port (ODO-1 from SGE, Ringwood, Australia) connected by a flow splitter to the column exit. The column was a DB-Wax from J&W (Folsom, CA), 30 m × 0.32 mm × 0.5 μm. The temperature program was as follows: 35 °C (1min), 5 °C min^{-1}, 210 °C. The injector and detector were both kept at 230 °C. The flow of air in the humidifier was 25 mL min^{-1}. One microliter of solution was injected in splitless mode, the splitless time being 1 min.

Panel 2: Analytical conditions were the same, except for the gas chromatograph, which was a HP 5890 II (Palo Alto, CA) equipped with a FID and a sniffing port ODP 2 (Gerstel, Mülheim an der Ruhr, Germany).

GC-O. *Panel 1:* The study was performed by five experienced judges (four women and one man, mean age 30 years, standard deviation 6 years). In the first series of sessions the judges sniffed during the whole run time without interruption; in the next series of sessions they were instructed to start sniffing 1 min before the expected retention time only and to stop 1 min after this time.

Panel 2: The study was performed by seven experienced judges (five women and two men, mean age 36 years, standard deviation 8 years). The judges were instructed to start sniffing 1 min before the expected retention time only and to stop 1 min after this time.

All judges were asked to estimate the overall intensity of each odor event using a 0−3 scale with seven possible scores (half-values allowed). The judges were not informed what kind of solution was in the humidifier. Humidifier solutions [water, 10 and 20% (v/v) hydroalcoholic solutions] were freshly prepared for each panelist and session. The type of solution was for each session selected according to Latin square design to randomize the effect of different ethanol levels.

Statistics. Because the intensity data were not normally distributed across the panels, medians were used for the calculation of panel intensities for each odorant. Nonparametric tests were used to check general trends: the Mann−Whitney rank sum test for comparison of the independent panels and the Kruskal−Wallis one-way analysis of variance on ranks for comparison of ethanol levels. Pairwise multiple comparisons were performed using Tukey and Student−Newman−Keuls methods.

RESULTS

Different Results Were Obtained Depending on the Methodology of the GC-O Design. In the standard, uninterrupted, GC-O design the increased level of alcohol had a rather suppressive effect on median panel intensities and frequencies of citation (**Tables 2** and **3**; "incessant sniffing"). Increase of panel intensity with introduction of solution into the humidifier was observed only for 2-methylbutanoic acid and α-ionone and partly for γ-octalactone, but some panelists ceased to perceive it at 20% alcohol level. The suppressing effect of mainly 20% ethanol is clear also from average (total) median intensities and frequencies of citation (**Tables 2** and **3**).

When the judges started to sniff a short time before the expected retention time, the suppression effect almost disappeared (**Tables 2** and **3**, "Target-oriented sniffing, Panel 1"). Decrease of panel intensity with increasing content of ethanol was observed only for *cis*-3-hexenal and methionol; decrease at 10% ethanol level appeared also for linalool. Total intensities and frequencies also confirm the enhancing effect of ethanol on the perceived intensity in the target-oriented design.

To confirm these interesting results, an independent panel performed the "target-oriented" design of GC-O, too (**Tables 2** and **3**, "Target-oriented sniffing, Panel 2"). A steady increase of panel intensity with rising concentration of ethanol in the humidifier was observed in eight cases. In two cases (linalool and α-ionone) the intensity increased at the highest ethanol level only. The exceptions were ethyl butanoate, for which the panel intensity dropped to zero with 20% ethanol in the humidifier, and 3-methyl-1-butanol, for which the panel intensity was lower in both designs with hydroalcoholic solutions. The differences in total intensities were even more pronounced than for the first panel (**Table 2**). The coherency of these results with the results obtained by the first panel was not absolute, as for *cis*-3-hexenal and methionol the trends were opposite. On the whole the responses of the panels were, however, compatible, as proved by the rank sum test with which no significant difference was found between them ($p > 0.1$).

The trend of citation frequency increase with rising content of ethanol in the humidifier (**Table 3**) was very clear for linalool, 2-methylbutanoic acid, and α-ionone in the first panel and for decanal and γ-octalactone in the second panel. This proves that at least for these compounds for certain judges the copresence of ethanolic vapors with the vapors of the analyte caused an increase of sensitivity of their sense of smell for the mentioned chemicals. It is also interesting to see the coherency of the two panels for 3-methyl-1-butanol (decrease of frequency of responses at the 10% level) and decanal (increase of frequency of responses at the 20% level).

We performed a nonparametric ANOVA to determine if the effect of alcohol on the intensity of panel response was statistically significant. The differences in the median values among the treatment groups were greater than would be expected by chance only in the second panel ($p < 0.05$). The Tukey pairwise test showed here a significant difference between the setup with no alcohol and 20% alcohol. The less conservative Student−Newman−Keuls test additionally found differences also between pure water and the 10% hydroalcoholic solution. The effect of ethanol level on panel intensity was more pronounced when the data sets from both panels were merged

Table 2. Medians of Individual Intensities of 12 Odorants at Three Levels of Ethanol in Humidifier[a]

	Incessant sniffing, Panel 1			Target-oriented sniffing, Panel 1			Target-oriented sniffing, Panel 2		
Compound	0% Ethanol	10% Ethanol	20% Ethanol	0% Ethanol	10% Ethanol	20% Ethanol	0% Ethanol	10% Ethanol	20% Ethanol
Ethyl butanoate	0	0	0	0	0	0	0.5	0.5	0
cis-3-Hexenal	1.5	1.5	1.5	2	1.5	1.5	1	**1.3**	**1.8**
2-Heptanone	1.5	1.5	1.5	1.5	1.5	**2**	1.5	**2**	**2**
3-Methyl-1-butanol	0	0	0	0	0	0	0.5	0	0.3
Decanal	1.5	0	0	1	1	**2**	1.3	**2.3**	**2.3**
Linalool	0	0	0	1.5	1	1.5	1	1	**1.5**
Acetylpyrazine	0	0	0	0.5	**1.5**	**2**	1	**1.5**	**1.8**
2-Methylbutanoic acid	0	**1**	**1**	0.5	**1**	**2.5**	0.5	**2**	**1.3**
Methionol	1	1	0.5	2	1.5	0.5	1.8	**2.5**	**2.8**
α-Ionone	0	**0.5**	**1**	1	**2**	**2**	1.5	1.3	**2.3**
γ-Octalactone	1	**1.5**	0	2	2	2	1	**2**	**2**
Eugenol	2	2	2	1.5	**2**	**2**	0.5	**0.8**	**1.3**
Average (total) intensity	0.7	**0.8**	0.6	1.1	**1.3**	**1.5**	1	**1.4**	**1.6**

[a] Increases against the control (0% ethanol) are highlighted in bold letters, decreases with gray background.

Table 3. Frequency of Odor Citations (Expressed as Percentage) in the Performed GC-O Analyses[a]

	Incessant sniffing, Panel 1			Target-oriented sniffing, Panel 1			Target-oriented sniffing, Panel 2		
Compound	0% Ethanol	10% Ethanol	20% Ethanol	0% Ethanol	10% Ethanol	20% Ethanol	0% Ethanol	10% Ethanol	20% Ethanol
Ethyl butanoate	40	40	0	20	**40**	20	57	57	43
cis-3-Hexenal	80	80	80	100	100	100	100	100	100
2-Heptanone	100	60	80	100	100	100	100	100	100
3-Methyl-1-butanol	0	0	0	40	20	40	71	43	71
Decanal	60	40	20	60	60	**80**	86	86	**100**
Linalool	40	40	40	60	**100**	**80**	100	100	100
Acetylpyrazine	40	20	0	80	80	80	100	100	100
2-Methylbutanoic acid	40	**80**	**60**	60	**80**	**80**	100	100	100
Methionol	60	60	60	100	80	60	100	100	100
α-Ionone	40	**60**	**80**	60	**100**	**100**	100	100	100
γ-Octalactone	100	100	40	100	100	100	86	86	**100**
Eugenol	100	100	100	100	100	100	100	100	100
Average (total) intensity	58	57	47	73	**80**	**78**	92	89	**93**

[a] Increases against the control (0% ethanol) are highlighted in bold letters, decreases with gray background.

($p < 0.01$). The results of pairwise tests from this ANOVA were the same as we found for the second panel alone.

DISCUSSION

The deep effect of ethanol on the perception of volatiles was, to our knowledge, observed for the first time by Williams (*9, 10*). Ethanol altered the flavor of alcoholic beverages and gave the products persistence and body. It had a mellowing and suppressing effect on the aroma and altered the acid sugar balance. In his later work (*10*), however, Williams found that a small quantity of ethanol enhanced the intensity of cider aroma. LeBerre et al. (*11*) also reported synergistic effect of ethanol on odor intensity of isoamyl acetate and whiskey lactone. Fischer and Berger (*12*) reported that dealcoholization of wine led to reduction of its fruitiness, whereas the vegetative, musty, and sweaty odors increased. In contrast, Guth (*13*) reported an increase of fruity character with reduced content of ethanol in Gewürztraminer wines. Similarly, Escudero et al. (*14*) observed a suppressing effect of ethanol on the fruity character of a mixture of esters dissolved in synthetic wine. Obviously, these antagonist results just show how different the individual sensory data sometimes can be (similar to what we observed for *cis*-3-hexenal and methionol). The reason for this behavior is out of the scope of this paper.

At higher ethanol levels in water (such as in wines or distillates) the volatiles are better dissolved and their partition toward headspace decreases (*11, 13, 15*), mainly for more polar compounds (*11, 15*). In the case of our GC-O setup, however, a quick mixing of vapors in the sniffing funnel was taking place, without a direct influence of water. To our knowledge only Guth performed a study comparable with the target-oriented analyses we made (*3, 13*), wherein he observed an increase of odor thresholds in air (measured with an olfactometer) with the introduction of ethanol vapors for three esters including ethyl butanoate and three alcohols including 3-methyl-1-butanol. These findings corroborate our results on 3-methyl-1-butanol in both panels and methionol in the case of the first and ethyl butanoate in the case of the second panel. The hydroxyl group seems to be a weak factor in the case of eugenol, the intensity of which increased in the presence of ethanolic vapors for both panels (**Table 2**). In addition, in the first panel the panel intensity of the aldehyde *cis*-3-hexenal also decreased with the presence of ethanol in the humidifier.

Knowing this, it is advisable to check the performance of each panel and explore the possible advantages from amplification of intensity. If one needs to quantify specific odors by GC-O (*5, 6*), it would be of great benefit to check how the panel will react on addition of ethanol into the humidifier. Amplification of panel intensity may considerably enhance confidence in the detection and recognition of the odors, yet increasing the sensitivity of the method. Using hydroalcoholic solutions seems to act unfavorably for the classical, incessant GC-O design.

Furthermore, one needs to keep in mind that the panelists are exposed to ethanol vapors for a relatively long time.

Our results may suggest that ethanol is competing with alcohols and esters on a perceptive receptor level, diminishing the ability to differentiate new odor from the background. On the contrary, for the remaining groups of compounds ethanol may act as a contrast-forming, focusing factor. Ethanol might also help to accumulate the odorants in the lipid-rich perireceptor mucosa of the *regio olfactoria*, similarly to its activity in hydroalcoholic solutions (*11*, *13*, *15*), and herewith promote activation of a wider array of olfactory receptors, possibly by temporal integration (*16*, *17*).

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