

Aroma Extract Dilution Analysis. Precision and Optimal Experimental Design

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The odor thresholds of 12 different compounds have been determined in an AEDA experiment using a panel composed of 8 individuals. Only in one case is the distribution of thresholds among judges significantly different from the log-normal. The cause of that departure from normality seems to be a cross adaptation rather than anosmia. The standard deviations (SD) range from $2^{0.7}$ to $2^{4.1}$, with $2^{1.8}$ as average. If the AEDA is carried out at a dilution rate, R , and dilution R^P (where $P = 0, 1, 2, \dots$) is the last one at which a compound was detected by a judge, the flavor dilution (FD) factor that should be provided for that judge is $R^{(P+0.5)}$. In the case where several judges carry out the AEDA, the FD for a given compound should be the geometric mean of the FD provided by all the judges. The SD of the distribution of FDs is related to that of the distribution of odor thresholds, but is strongly influenced by the dilution rate, R , being higher with higher R values. The relationship between both SDs can be used to determine the expected precision (measured as the geometric length of the 95% confidence interval) of the result of an AEDA experiment as a function of the dilution rate, the number of judges, and the SD of the distribution of thresholds. Different simulations have shown that in most cases, a dilution rate of 10 is the best option, and that lower dilution rates are advantageous only if the analyzed solution contains compounds with a very narrow distribution of thresholds.

KEYWORDS: Aroma extract dilution analysis; flavor dilution factor; GC–olfactometry; odor thresholds; precision; optimization

INTRODUCTION

Gas chromatography–olfactometry (GC–O) has become the most widely used technique for evaluation of complex food flavors (I) because it directly provides important information about the presence of compounds with aromatic properties in a foodstuff. Generally speaking, the main purpose of the GC–O research is to list and order the aroma compounds present in the foodstuff according to their potential importance in the food flavor. The way in which these lists are built differ among the different GC–O techniques, which can be classified into the three following broad categories: (1) based on determination of threshold concentration (aroma extract dilution analysis (AEDA) (2, 3), CHARM (4)); (2) based on the measurement of the frequency of citations (5); and (3) based on the assessment of intensity (OSME (6, 7), cross-modality matching (8), or flavor impact values (9)).

Despite many criticisms (nonconformity with psychophysical laws, no correction for losses of odorants during isolation procedure), AEDA is the most frequently used method for the screening of flavor impact compounds, probably because of its

simplicity. According to this technique, the flavor extract is sequentially diluted (following a rate R , where R is usually 2, 3, 5, or 10) and each dilution is analyzed by GC–O by a small number of judges. The flavor dilution (FD) of an odorant corresponds to the maximum dilution at which that odorant can be perceived by at least one of the judges. Numerically, if the last dilution at which compound c was perceived was P (where P usually is 0, 1, 2, ..., n), its FD is R^P . When several judges are used in a study, usually the maximum FD is provided as the FD factor of that compound.

In most cases, AEDA is just the first step in a long research program in which the actual importance of the odorants in the foodstuff is assessed with suppression and reconstitution tests (10, 11). In this context it is assumed that FD factors are just rough estimations of the importance of an odorant and, consequently, little attention has been paid to the different alternatives for computing FD factors and their corresponding analytical figures of merit. This can explain why, despite being the most frequently used GCO technique, and in contrast to the other GCO strategies, no statistical evaluation of AEDA data has ever been performed. However, there are several situations in which FD factors are being given a quantitative value, such as the technique known as comparative AEDA (12–14), in which similar weights of two or more products are extracted in a similar way and analyzed by AEDA under equivalent

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Table 1. Solution Used in the Study (Composition, Retention Times, and Basic AEDA Results)

| compound | retention time | initial concentration (mg/L) | min FD | max FD | average FD |
|-----------------------|----------------|------------------------------|--------|--------|------------|
| ethyl butyrate | 5 min 1 s | internal standard | | | |
| 2-methylbutyrate | 5 min 27 s | 1.88 | 1 | 64 | 4 |
| 3-methylbutyrate | 5 min 45 s | 10.8 | 8 | 512 | 128 |
| linalool | 18 min 40 s | 10.1 | 8 | 32 | 16 |
| isobutyric acid | 19 min 11 s | 199 | 1 | 32 | 9.5 |
| methyl benzoate | 20 min 30 s | 198 | 2 | 32 | 7.3 |
| isovaleric acid | 21 min 45 s | 2.50 | 2 | 128 | 8.7 |
| β -damascenone | 25 min 20 s | 0.080 | 1 | 128 | 12.3 |
| α -ionone | 26 min 5 s | 9.70 | 1 | 4 | 2.8 |
| t-whiskeylactone | 28 min 40 s | 3.98 | 1 | 64 | 5.7 |
| 4-ethylguaiaicol | 29 min 59 s | internal standard | | | |
| ethyl cinnamate | 32 min 8 s | 78.2 | 1 | 1024 | 117 |
| γ -decalactone | 32 min 26 s | 1.41 | 1 | 16 | 3.1 |
| eugenol | 32 min 47 s | 9.70 | 4 | 128 | 16 |

conditions to ensure similar recoveries. Other authors have put the emphasis on the production of extracts retaining the odor characteristics of the original product as much as possible, so that odor and flavor properties of the food can be extrapolated from conclusions based on FD factors from the extract (15–19). Finally, some authors have developed strategies that allow the direct GC–O study of the headspace on the foodstuff (5, 20), and therefore, the FD factor of an odorant estimated following this approach should be a direct measure of its potency in the headspace of the product. It is clear that in all these previous cases, some knowledge about the shape and value of the confidence intervals of the FD factors is required to extract quantitative conclusions based on these measurements.

The main purpose of the present paper is to review the theoretical framework beneath the concept of FD factor in order to assess how they must be calculated, and how different operative conditions (number of judges and rate of dilution) affect their confidence intervals and, therefore, their usefulness in comparative or quantitative studies.

MATERIALS AND METHODS

Determination of Odor Thresholds. A standard solution containing the compounds at the concentrations indicated in **Table 1** was prepared (all standards were purchased from Sigma-Aldrich Química, Madrid, Spain). The solvent of that solution was dichloromethane (Fisher Scientific, Leicester, UK).

Judges were selected because of their interest and availability. All of them had had previous experience in GC–O tests. After the training period (2 weeks) in which the judges were instructed to smell different test solutions containing up to 20 different aroma chemicals, all the judges were able to make repetitive analyses of the test solutions. The panel finally selected was composed of 8 individuals (5 females and 3 males) whose ages ranged from 25 to 45.

Gas Chromatography–Olfactometry (GC–O). A Fisons 8360 gas chromatograph equipped with a polar fused silica column (J&W DB-Wax (30 m \times 0.32 mm \times 0.5 μ m)) was used. The solutions were injected in splitless mode (injector at 250 °C), and the compounds were separated using the following oven program: 40 °C (3 min), 5 °C/min, 200 °C (8 min). The column flow was split 1:1 at the column outlet between a FID detector (250 °C) and the olfactometric port (ODO-1 from SGE, Australia). To prevent condensation of high-boiling compounds on the port, this was heated sequentially using a laboratory-made rheostat (a hot wire) to 90 °C at 80 °C oven temperature, to 140 °C at 120 °C, and to 200 °C at 180 °C oven temperature.

The odor thresholds of the compounds were determined, following as close as possible, the normal AEDA practice. The standard solution was sequentially diluted 1:2, and the different dilutions were injected at random in the GC–O system. The FD factors obtained in this experiment can be seen in **Table 1**.

Table 2. GC–O Threshold Data (Mean Values, Standard Deviations, Skewness, and Kurtosis of their Distributions)

| compound | threshold | standard deviation | N | skewness | kurtosis |
|-----------------------|--------------------------|--------------------|---|------------------|------------------|
| 2-methylbutyrate | $2^{-1.1} = 0.47$ mg/L | $2^{2.1}$ | 8 | −1.1 | 0.81 |
| 3-methylbutyrate | $2^{-3.6} = 0.084$ mg/L | $2^{1.8}$ | 8 | 1.6 ^a | 3.2 ^a |
| linalool | $2^{-0.66} = 0.63$ mg/L | $2^{0.76}$ | 8 | 0.0 | −0.70 |
| isobutyric acid | $2^{4.4} = 21$ mg/L | $2^{2.4}$ | 8 | 0.14 | −1.8 |
| methyl benzoate | $2^{4.8} = 27$ mg/L | $2^{1.5}$ | 8 | 0.03 | −1.9 |
| isovaleric acid | $2^{-1.8} = 0.29$ mg/L | $2^{2.1}$ | 8 | −0.89 | 0.08 |
| β -damascenone | $2^{-7.3} = 0.0065$ mg/L | $2^{3.0}$ | 8 | 0.02 | −1.5 |
| α -ionone | $2^{1.8} = 3.4$ mg/L | $2^{1.1}$ | 8 | 0.47 | −0.83 |
| t-whiskeylactone | $2^{-0.51} = 0.70$ mg/L | $2^{1.8}$ | 8 | −0.82 | 1.8 |
| ethyl cinnamate | $2^{-0.59} = 0.67$ mg/L | $2^{4.1}$ | 8 | 0.85 | −0.8 |
| γ -decalactone | $2^{-1.13} = 0.46$ mg/L | $2^{1.7}$ | 8 | −1.0 | 1.5 |
| eugenol | $2^{-0.72} = 0.61$ mg/L | $2^{1.5}$ | 8 | −0.33 | −1.5 |

^a Significant departure from the log-normal distribution.

Treatment of Data. All the data treatments and simulations have been carried out with an Excel spreadsheet.

RESULTS AND DISCUSSION

Distribution of Odor Thresholds in GC–O. According to the general scientific literature on olfactometry, odor thresholds follow a log-normal distribution among healthy people (21, 22), although in some cases a slight skew in the right part of the distribution has been noted (22). The most important exceptions to this behavior are due to the presence of anosmias, but according to data reported by Punter (22) and Marin et al. (23), these are not very frequent among healthy people. Leaving aside well-documented cases of bimodal distributions (24–26), the log-normal distribution can be taken as a good approximation. According to data by Punter, the standard deviation (SD) of the corresponding distributions range from $2^{0.78}$ (toluene) to $2^{5.31}$ (tetrachloromethane), with $2^{2.0}$ as the most frequent value. This means that the 95% confidence intervals range between 1/3 and 3 times the mean (in the case of toluene), between 1/1500 and 1500 times the mean (tetrachloromethane), and between 1/16 and 16 times the mean in the most frequent case. These results are in agreement with the conclusions from Amoore, which stated that in most cases 95% confidence intervals lie between 1/10 and 10 times the mean; equivalent to $SD = 2^{1.7}$ (21, 27).

Other authors working specifically on GC–O found smaller SDs for the mean thresholds: between $2^{0.3}$ and 2^1 (23, 28). This discrepancy could be due to the fact that those authors used smaller panels of trained people selected by stringent criteria. In fact, Marin et al. report that 50% of their potential judges were rejected during the selection process, which is equivalent to shrinking the original SD by a factor 3.

We have determined the olfactometric thresholds of twelve compounds for a panel of 8 trained people in an AEDA experiment. The mean thresholds, the SDs, and some other parameters from the observed distributions are given in **Table 2**. Data in that table show very good agreement with the basic results from Amoore or Punter. The SDs range from $2^{0.76}$ to $2^{4.1}$, with $2^{1.82}$ the median. In addition, and attending to the Skewness and Kurtosis of the distributions, only in the case of 3-methylbutyrate did the distributions showed a significant departure from the log-normal distribution. The cause of this was not a judge suffering from a specific anosmia, but rather an adaptation effect caused by the previous elution of 2-methylbutyrate. Leaving aside this compound, the “a posteriori” elimination of the least sensitive or least reproducible judges did not improve the results in the rest of the cases. These results

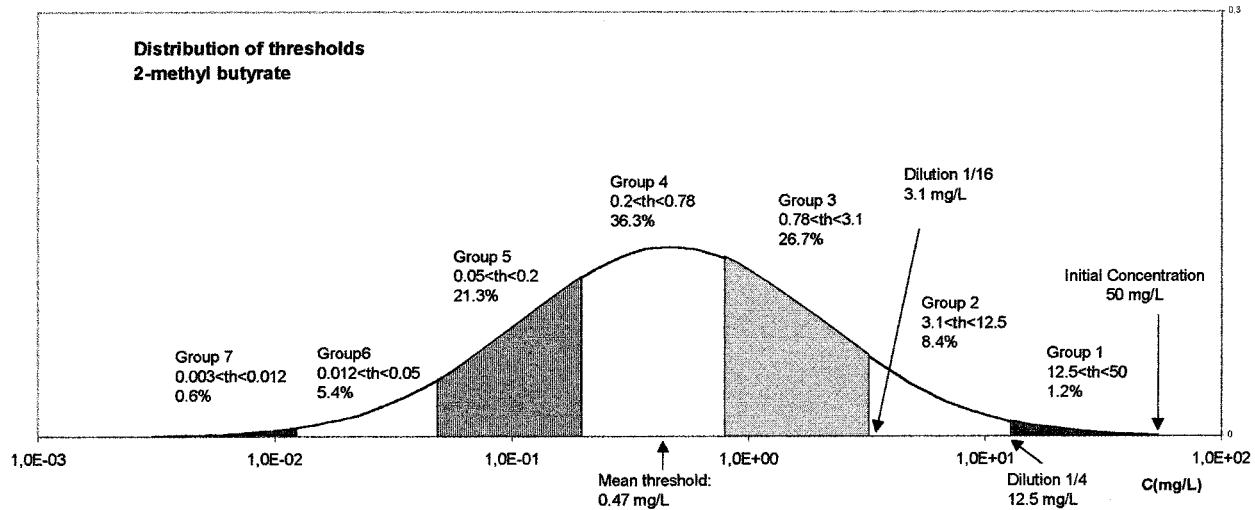


Figure 1. Distribution of thresholds for 2-methylbutyrate, and groups into which the population of judges is divided, in the AEDA analysis of a 50 mg/L solution.

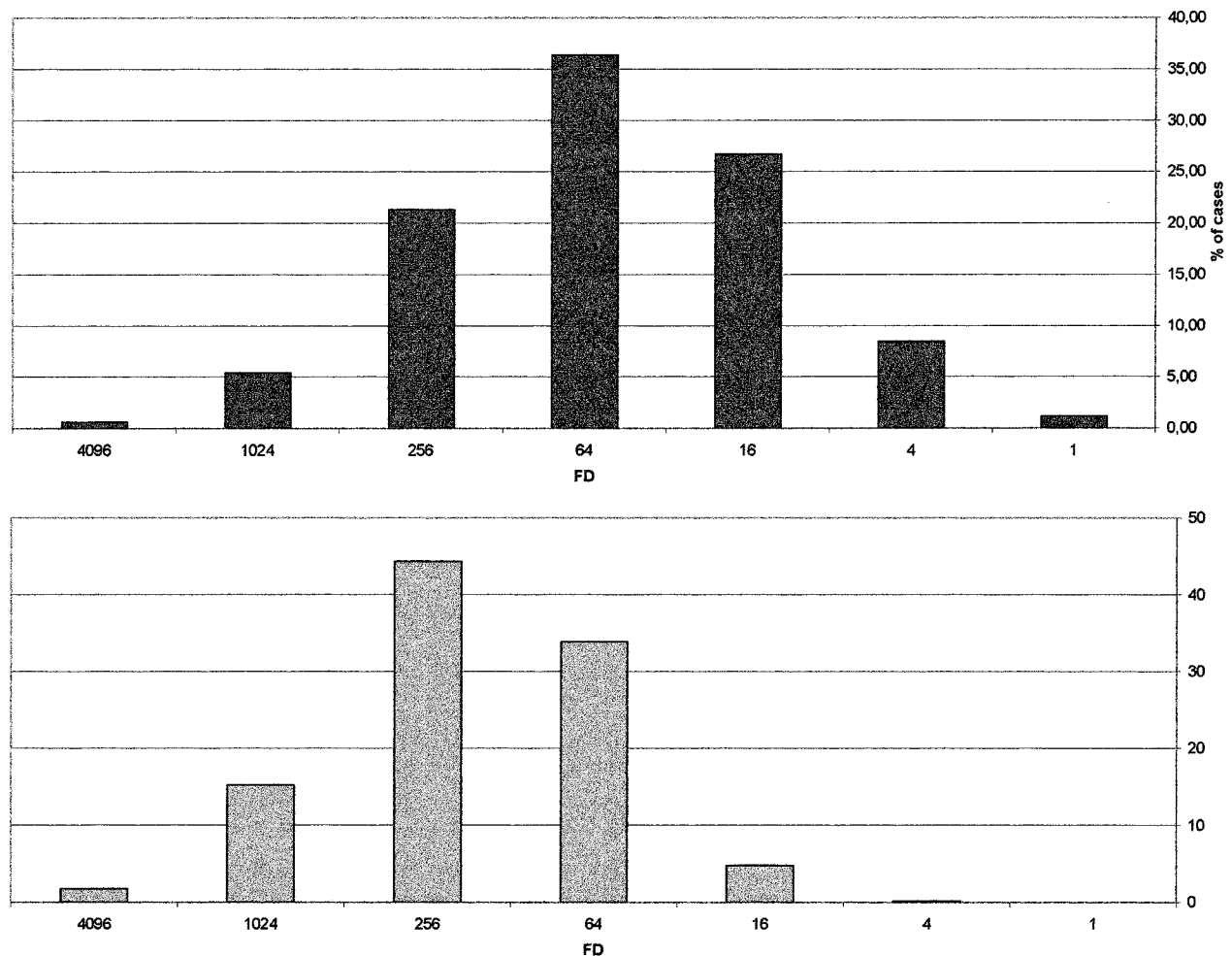


Figure 2. Biased distributions of FDs potentially obtained in the AEDA analysis of a 50 mg/L solution of 2-methylbutyrate. (a) Distribution of individual FDs. If the judge fails in the detection of the odorant at the dilution $P + 1$, the FD given is R^P ; (b) distribution of FDs from a panel of three judges; if the individual FDs are R^{P1} , R^{P2} , and R^{P3} , the FD given is the maximum of the three values.

allow us to state that the assumption of a log-normal distribution of GC-O odor thresholds is correct and that SDs in the range $2^{0.7} - 2^4$ are expected.

Giving a Flavor Dilution Factor for a Judge. If a judge has been able to detect the odor at the dilution R^P and has failed in its detection at the dilution R^{P+1} , the FD usually given for

that odor and judge is R^P , with R being the dilution rate. This FD is systematically lower than the expected FD, as it will be shown.

Let us suppose that we are doing an AEDA study of a solution containing 50 mg/L of 2-methylbutyrate at a dilution rate $R = 4$. The mean threshold of this compound is 0.47 mg/L and its

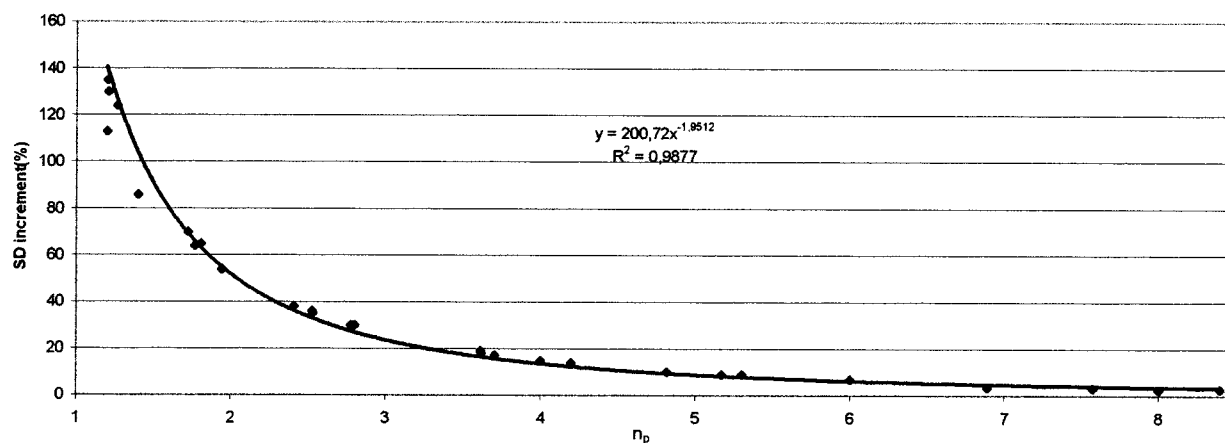


Figure 3. Relationship between the number of potential FDs obtained in an AEDA experiment (n_p) and the increment (as %) in the SD of the distribution of FDs related to the SD of the distribution of thresholds. Both SDs are expressed as the exponents of 2.

SD is $2^{2.1} = 4.29$ mg/L (see **Table 2**). The corresponding distribution of thresholds can be seen in **Figure 1**. The AEDA experiment divides the whole population into the 7 groups marked in the figure. Group 1 is formed by the 1.2% of the population that is able to detect the initial solution at 50 mg/L, but not its $1/4$ dilution. Group 2 is formed by the 8.4% of people able to detect the $1/4$ dilution but not the $1/16$, and so on for the rest of the groups. According to the aforementioned criteria for giving FDs, group 1 will be given a FD of 1, group 2 will be given a FD of 4, group 3 will be given a FD of 16, group 4 will be given a FD of 64, and so on. It can be noted that this implies that we assume the odor threshold that best represents each one of the groups is not the mean for that group but the maximum threshold (of that particular group). The corresponding distribution of FDs that we would obtain is shown in **Figure 2a**. As expected, the geometric mean of that distribution is not the real FD of the solution ($50/0.47 = 106$), but 53. This value is displaced $4^{-0.5}$ geometric units (i.e., $106/2$) from the real center of the distribution. In general, if the AEDA experiment is carried out at a dilution R , the mean FD will be displaced $R^{-0.5}$ geometric units from the expected FD. The bias introduced, therefore, amounts to

$$\text{bias}(\%) = \left(\frac{1}{\sqrt{R}} - 1 \right) \times 100 \quad (1)$$

This effect is corrected if the FD given for the group of people able to detect the compound at a dilution R^P and failing in the detection at the dilution $R^{(P+1)}$, is $R^{(P+0.5)}$ instead of R^P . For the same reason, if a judge fails in the detection of the odorant in the most concentrated extract (R^0), a FD of $R^{(-0.5)}$ should be provided. It can be argued that this operation is only a rescaling that complicates calculations, however, it will make comparison of FDs possible even if the dilution rates are different. In general, if the SD of the distribution of odor thresholds is known, it follows that the confidence intervals for the FDs from a judge are $R^{(P+0.5 \pm 2SD)}$, provided that the dilution rate chosen is small, as it will be shown later.

FDs in Experiments with More Than 1 Judge. There are no clear criteria about how to calculate FDs in the case in which the AEDA experiment is performed by two or more judges (29). In fact, this information is missing from nearly all the papers on AEDA due, probably, to the reasons cited in the Introduction. As AEDA was first devised as a screening technique, a logical option for a given compound was to choose as its FD the maximum of all the FDs obtained by the different judges. Of

Table 3. Influence of the Dilution Rate and the SD of the Distribution of Thresholds on the SD and Central Values of the Distributions of FD Obtained in the AEDA Analysis of a Solution with an Odorant with FD True Value Set at 106

| R | | SD of the distribution of thresholds | | | | | |
|-----|---------|--------------------------------------|------------|---------------|------------|---------------|---------------|
| | | $S = 2^4$ | $S = 2^3$ | $S = 2^{2.1}$ | $S = 2^1$ | $S = 2^{0.7}$ | $S = 2^{0.3}$ |
| 2 | n_p^a | 16 | 12 | 8.4 | 4.0 | 2.8 | 1.2 |
| | mean | 110 | 106 | 106 | 106 | 106 | 102 |
| | SD | $2^{3.6}$ | $2^{3.0}$ | $2^{2.17}$ | $2^{1.15}$ | $2^{0.91}$ | $2^{0.64}$ |
| 3 | n_p | 10.1 | 7.6 | 5.3 | 2.5 | 1.8 | 0.8 |
| | mean | 110 | 106 | 106 | 106 | 107 | 126 |
| | SD | $2^{4.0}$ | $2^{3.1}$ | $2^{2.29}$ | $2^{1.36}$ | $2^{1.15}$ | $2^{0.92}$ |
| 4 | n_p | 8.0 | 6.0 | 4.2 | 2.0 | 1.4 | 0.6 |
| | mean | 109 | 106 | 106 | 106 | 109 | 127 |
| | SD | $2^{4.1}$ | $2^{3.2}$ | $2^{2.4}$ | $2^{1.52}$ | $2^{1.30}$ | $2^{1.01}$ |
| 5 | n_p | 6.9 | 5.3 | 3.6 | 1.7 | 1.2 | 0.5 |
| | mean | 109 | 106 | 106 | 105 | 101 | 79 |
| | SD | $2^{4.14}$ | $2^{3.28}$ | $2^{2.49}$ | $2^{1.7}$ | $2^{1.61}$ | $2^{1.51}$ |
| 10 | n_p | 4.8 | 3.6 | 2.5 | 1.2 | 0.8 | |
| | mean | 108 | 106 | 106 | 105 | 112 | |
| | SD | $2^{4.39}$ | $2^{3.56}$ | $2^{2.85}$ | $2^{2.35}$ | $2^{2.34}$ | |
| 20 | n_p | 3.7 | 2.8 | 1.9 | 0.9 | | |
| | mean | 107 | 106 | 106 | 94 | | |
| | SD | $2^{4.68}$ | $2^{3.9}$ | $2^{3.23}$ | $2^{2.3}$ | | |
| 100 | n_p | 2.4 | 1.8 | 1.3 | | | |
| | mean | 106 | 106 | 108 | | | |
| | SD | $2^{5.54}$ | $2^{4.94}$ | $2^{4.71}$ | | | |

^a n_p is the quotient between the logs of the 95% confidence interval of the true odor threshold and of R , the dilution rate.

course, this makes sense as long as AEDA data are not given any quantitative value, because this criterion gives strongly biased results. This is shown in **Figure 2b**, which presents the hypothetical distribution of results obtained if 3 judges analyze the 50 mg/L 2-methylbutyrate solution and the aforementioned criterion to select FDs is used. The center of the distribution is now 181, higher than the expected value. In addition the distribution is no longer symmetric, and accordingly, confidence intervals are ill defined.

A more convenient choice for the FD factor is, of course, the geometric mean of all those provided for a compound by the different judges. In general, if the FDs from the n different judges are $R^{(P_1+0.5)}$, $R^{(P_2+0.5)}$, ..., the FD for the panel will be $R^{((P_1+P_2+\dots)/n+0.5)}$, or $R^{(P+0.5)}$, with P being the arithmetic mean of P_i . If the SD of the distribution of odor thresholds is known, the confidence intervals for that mean are $R^{(P+0.5 \pm 2SD/n^{1/2})}$ (formula valid only if R is small; SD must be given as the

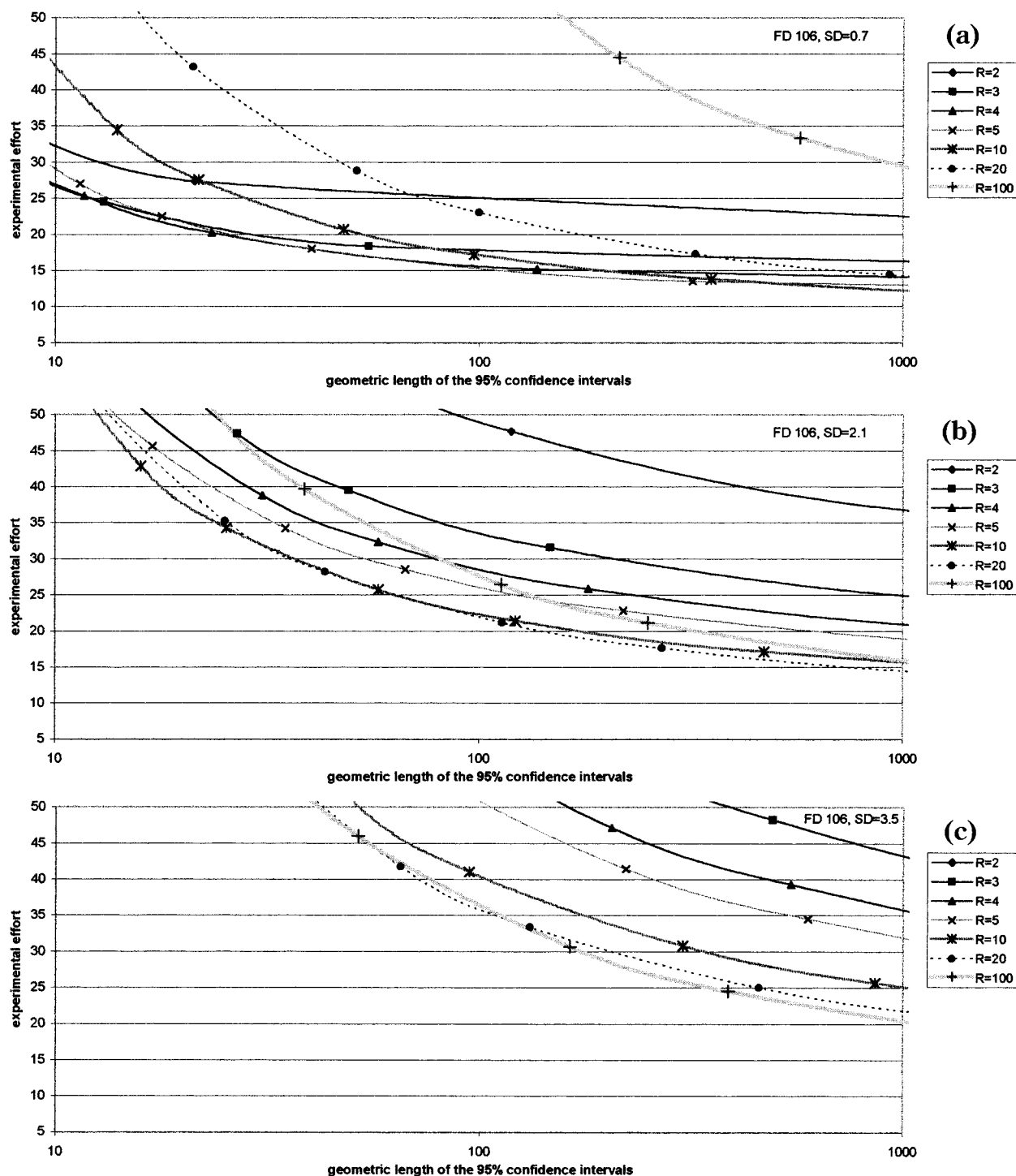


Figure 4. Experimental effort (number of judges \times number of tests per judge) required to achieve a given precision (expressed as the geometric length of the 95% confidence intervals of the mean) in the AEDA analysis of a compound at a concentration 106 times higher than its threshold and whose odor threshold distributes with (a) $S = 2^{0.7}$; (b) $S = 2^{2.1}$; (c) $S = 2^{3.5}$.

exponent of R). If the SD of the distribution of odor thresholds is not known, and SD' is the standard deviation of the P_i obtained by the different judges, the confidence intervals are $R^{(P+0.5 \pm tSD'/n^{1/2})}$ where t has $n - 1$ degrees of freedom.

Influence of the Dilution Rate. The dilution rate influences the number of possible FD factors (n_p) obtained in a given experiment because it determines the number of groups in which the overall population is separated, as it was shown in Figure 1. The higher the R , the lower the number of groups. This parameter can be computed with the following formula:

$$n_p = \frac{\log\left(\frac{C_{\text{Max}}^{\text{threshold}}}{C_{\text{Min}}^{\text{threshold}}}\right)}{\log(R)} = \frac{\log\left(\frac{R^{\text{mean}+2SD}}{R^{\text{mean}-2SD}}\right)}{\log(R)} = 4SD(\text{expressed as exponent of } R) \quad (2)$$

The max and min thresholds can be arbitrarily set at mean \pm 2SD (95%). The number of possible FD factors in the distribu-

tion of potential results is important because the reconstitution of the distribution function with few points is not satisfactory. On the contrary, too many results require a huge experimental effort. The effect that n_p has on the properties of the distribution of FD factors is shown in **Table 3** and in **Figure 3**.

Results in the table show that the number of possible results affects primarily the standard deviation of the mean and, secondarily, the value of the mean itself. With $8 > n_p > 4$ the standard deviation is only 2–15% higher than that of the original distribution. With $4 > n_p > 2.6$ the standard deviation is 10–30% higher, whereas if $2.6 > n_p > 1.3$ the standard deviation is 30–100% higher than that of the original distribution. This effect is shown and modeled in **Figure 3**, which shows the relationship between n_p and the increment in SD. The function represented in the figure allows us to predict the standard deviation that, on average, will be obtained when the FD of an odorant whose threshold distributes with a given SD is calculated at a given dilution rate (SD expressed as exponent of 2).

$$SD_{FD} = SD_{\text{threshold}}(1 + 2.0072n_p^{-1.9512}) \quad (3)$$

It can be seen in the table that for $n_p < 1.0$, not only is the standard deviation more than 150% of the original, but the center of the population is no longer that of the original distribution. All this means that if R is too high (and n_p is too low), it will be impossible to determine accurately the true FD of an odorant, even if thousands of judges are used. This also shows, that for $n_p < 8$, the confidence intervals of the mean P are $R^{(P+0.5 \pm 2SD/n^{1/2})}$, where **SD** is the SD of the distribution of the thresholds corrected by the factor given by eq 3, expressed in this case as exponent of R . If SD is not known, SD' can be used instead if 2 is replaced by the corresponding t factor.

Optimization of the Experimental Effort. The previous results can help us to understand how the design of the AEDA operation influences the precision of the results, and which strategies can lead to optimum results. Let us suppose that we are analyzing a solution containing 50 mg/L of 2-methylbutyrate. If we set dilution rate, R , at 2, it is easy to see that the most sensitive judges (whose threshold is around 0.026 mg/L) will detect the odorant along 11 dilutions (from dilution 0 to dilution 10), and will fail at the 12th experiment (dilution 11). If all the judges carry out the same number of experiments, the experimental effort is $12 \times n$, where n is the number of judges. In general, the experimental effort can be calculated with the following formula:

$$\text{Experimental Effort} = \left(\frac{\log\left(\frac{C}{C_{\text{Min}}^{\text{threshold}}}\right)}{\log(R)} + 1 \right) \times n \quad (4)$$

The expected standard deviation will be $2^{2.07 \times 1.0325} = 2^{2.137}$, according to the value of n_p and to eq 3. The 95% confidence interval will then be $2^{2t(SD/n^{1/2})}$, where t has $n - 1$ degrees of freedom and SD is expressed as exponent of 2. Therefore, if we use two judges to carry out the experiment, the average experimental effort will be 23.7 and the geometric length of the confidence intervals will be $3.6 \cdot 10^{11}$. If, instead, we use $R = 4$ and four judges, the experimental effort will be 25.7, and the length of the confidence intervals can be calculated as follows:

$$\text{Expected SD: } SD_{FD} = 2^{2.07 \times (1 + 2.0072 \times 4 \cdot 14^{-1.9512})} = 2^{2.33} = 4^{1.165}$$

$$\text{Confidence intervals: } 4^{2t(SD/n^{1/2})} = 4^{2 \times 3.18 \times (1.165/4^{1/2})} = 4^{3.70} = 170$$

which is clearly more efficient. The dependence between the experimental effort and the length of the confidence intervals for this case is plotted in **Figure 4b**. **Figures 4a** and **4c** also express this dependence for compounds for which the standard deviations of thresholds are 0.7 and 3.5, respectively. The general patterns of the plots do not change with concentration, and thereby, the three plots can be taken as a general guide for method optimization.

The figures show that for those compounds whose thresholds have a narrow distribution (**Figure 4a**), the most efficient strategies are dilution rates 4, 5, and even 10. In these cases it is possible to have quite narrow confidence intervals (1 order of magnitude) with a relatively low experimental effort. For instance, 5 judges testing five 1:4 dilutions each, of a compound for which the expected FD is 100, will provide a confidence interval of 11.6 geometric units.

For those other compounds whose distribution of thresholds has a SD around 2^2 (**Figure 4b**), the most efficient strategies are the use of dilution rates 10 and 20. In the case of a compound with expected FD 100, 6 judges testing four 1:10 dilutions each will provide a confidence interval of 55 geometric units.

Finally, for those other compounds for which the threshold has a very wide distribution, dilution rates of 20 and 100 are preferred, as can be seen in **Figure 4c**. Of course in these cases it is not possible to get narrow confidence intervals unless the experimental effort is very big.

All these results are not surprising. If the SD of the distribution of thresholds is narrow, there is not a big benefit on using a big sensory panel and it is better to use lower dilution rates. On the contrary, if the distribution of thresholds is very wide, it is better to use a large R and a bigger sensory panel.

Complex samples contain compounds with a wide range of FDs and, probably, with different distributions of thresholds, and, therefore, there is no single optimal solution. **Figure 4** suggests, however, that unless all compounds in the sample have very narrow distributions, a dilution rate of 10 seems to be a reasonable general compromise because it provides the highest efficiency at SDs between $2^{1.3}$ and $2^{2.5}$, the most frequent cases, and a satisfactory behavior at both narrow and wide distributions. On the other hand, if a minimum precision must be guaranteed for all the odorants detected in an AEDA experiment, a dilution rate of 20 may be preferred, as this rate provides maximum efficiency for the odorants with highest SD; although, according to data in **Table 2** and in reference 22, these cases are less frequent. For instance, a panel of 9 judges testing three or four 1:20 dilutions (the last dilution will have to be studied only by 2 or 3 more sensitive judges) is the least expensive approach to ensure confidence intervals narrower than 100 geometric units for all cases.

ABBREVIATIONS USED

AEDA, aroma extract dilution analysis; FD, flavor dilution factor; GC–O, gas chromatography–olfactometry; R , dilution rate; SD, standard deviation; P , number of the sequential dilution of the extract; n_p , number of possible FD results; n , number of judges.

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