Rapid aroma analysis and data interpretation using on-line mass spectrometry and visualisation software

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Abstract

Before starting detailed flavour analyses of food or beverage samples, it is often worthwhile carrying out a pre-screen to indicate what kinds of differences are present. Typically, the analyst wants to know: are there different compounds present / missing between samples or are the flavour profiles similar, but just differ in the amounts present and to what extent? With this knowledge, they can then design suitable analyses to investigate these differences more thoroughly. Here we present an example of a set of coffee samples, with different sensory aroma profiles, which were subjected to rapid headspace analyses using on-line APCI-mass spectrometry and the data imported into a visualization package to carry out data quality checks as well as some preliminary data interrogation. This approach yielded useful information in a few days, so that the next analytical steps could be planned, based on the pre-screen evidence.

Introduction

Modern analytical instruments can analyse complex flavour profiles effectively and provide qualitative and quantitative data on the compounds present (identity and amount). Techniques like GC-MS and LC-MS can deliver detailed information on the odour and tastant profiles, although the process can be time-consuming if full identification and quantification are carried out using LRI, spectral matching and authentic standards. Flavour scientists have recognised this issue already and have introduced techniques like GC-olfactometry [1] to filter the data so that, in this case, identification and quantification will focus only on the odorous compounds and therefore reduce the workload. Another approach is to compare the odour or taste profiles of samples under investigation to determine the nature of the differences and then choose which detailed analyses would be most suitable to characterise the samples. For example, if the analytical odour profiles of the samples are similar, then an obvious step would be to compare the taste profiles to determine what differences lay there. If the same odour compounds were present, but in quite different amounts, then it would be useful to consider the odour threshold values and determine, first whether the compound was present above the odour threshold value, and then, whether the change in amount could cause a significantly different sensory response using techniques like AEDA or Odour Activity Values (OAV) [2]. Pre-screening can provide evidence on which to base an experimental plan and, when coupled with data visualisation, it provides a way to handle all the data, carry out data quality checks, investigate the nature of the differences and produce results in 2D and 3D formats to clearly visualise the differences found in the data set. When full analyses become available, (compound identification, GC-O, odour threshold or sensory data) they can be added to the data table and the multi-factorial data can be subjected to interrogation using various statistical tests or by considering the relationships between chemical compounds. This latter approach can give an insight into the chemistry that occurred in the processes used to manufacture the food or the beverage and might be useful in understanding why the differences occurred.

B. Siegmund & E. Leitner (Eds): Flavour Sci., 2018, Verlag der Technischen Universität Graz
DOI: 10.3217/978-3-85125-593-5-95, CC BY-NC-ND 4.0

Experimental

Samples

Five coffee samples from Brazil (3), Ethiopia (1) and Vietnam (1) were provided by a coffee manufacturer. Samples were characterised by their lightness (L value) and origin only. Brewed coffee was prepared using 11 g of ground coffee and 240 mL of boiled water in a glass bottle fitted with a screw-top lid, providing a sampling port from which headspace was sampled at 65˚C.

Headspace analysis

A heat-jacketed, fused-silica transfer line (0.53 mm diameter, Agilent) was maintained at 150˚C and connected to the glass bottle containing the brewed coffee. The APCI-MS venturi inlet introduced a flow of headspace (10 mL/min) into the APCI source for 0.5 min. Triplicate samples were analysed.

APCI-MS conditions

A Micromass ZQ mass spectrometer fitted with a gas phase APCI interface (Waters, Manchester, U.K.) was operated as described previously [3]. Raw ion abundance and *m/z* values were recorded in a table, with no data processing.

Data wrangling

APCI data were imported into Spotfire (v7.9, Tibco, Palo Alto, USA) and extra identity tags (Sample) and calculated values (mean, standard deviation and %CV) were added. Data were un-pivoted to make columns that were appropriate for data filtering (*m/z*, abundance, sample, replicate, country of origin, lightness, mean, SD and %CV). JMP (v13, SAS Institute Inc., Cary, USA) was used for PCA plots.

Results and discussion

Data quality checks

The first step in any data analysis is to check and clean the data. The filter on the data visualisation screen (Fig 1a) shows that the APCI ion abundance values ranged from 4 to 40 x 10^6. In all analyses, there is a minimum signal to noise ratio and with knowledge of the analytical procedure being used, the minimum signal can be reset to remove spurious signals. In this case, the minimum acceptable APCI signal is >1000 so the slider was set to 1000, which excluded all values <1000 (Fig. 1b).

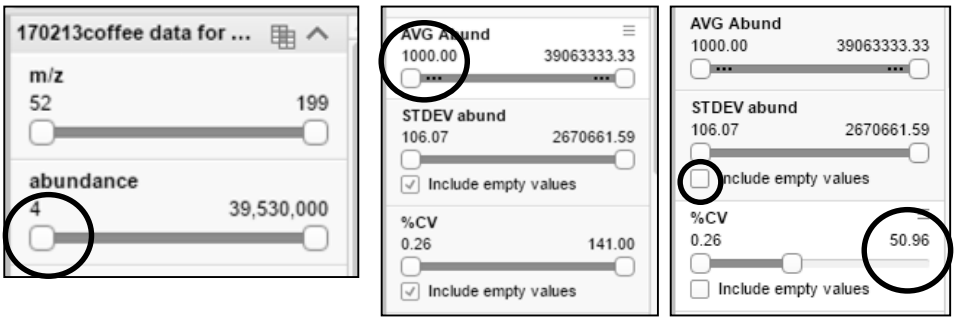

Figure 1: Screenshots of the filters in Spotfire and their adjustment to achieve rapid data cleaning. Circles indicate the changes made in steps 1a to 1c (left to right)

The Standard Deviation (Ion Abundance) filter (Figure 1b) also showed that there were some empty values in the data table; these occur when there are insufficient replicates to calculate a value, i.e. only one or two of the replicates showed the presence of that particular ion. These data can be removed by unclicking the "Show empty values" box to further clean the data. Finally, the %CV values show how robust the data are. Again, with knowledge of the samples and the typical analytical variation, the analyst can make informed choices about the degree of data variability they are willing to include in the data table and a final data cleaning step can be applied by adjusting the slider, in Fig 1c it is set at 50.96% but 24% was used to clean the data for PCA analysis. Using this feature of the visualisation software, the data can be cleaned by changing the filter box values to produce a robust data table which can then be interrogated with confidence.

Qualitative differences in coffee aroma profiles

A bar chart plot was constructed using Samples and *m/z* values on the X axis and Average Abundance on the Y axis. (Fig 2). Qualitative differences in the profile of the coffees were identified visually and, by hovering the cursor over each bar in Spotfire, the information about that point was displayed and assessed. Visual inspection led to two main conclusions. One was that the ion profiles of the samples in Fig. 2 were very similar, it was the amounts that were different. The other was that all the coffee profiles contained ions from *m/z* 50 to 200, so chromatographic conditions should be chosen to resolve compounds across this molecular weight range and, for quantification, internal standards would need to be added across the molecular weight range.

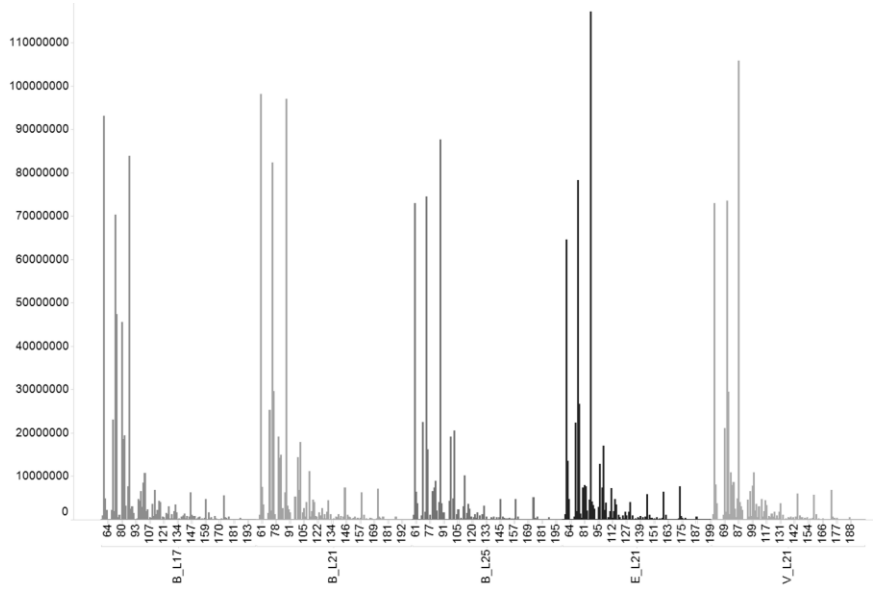

Figure 2: Ion profiles for each of the *m/z* values in the five coffee samples (Ion abundance are aggregated values for the three replicates)

Principal component analysis of the data using correlation and covariance matrices indicated that covariance, using the absolute data, not the normalised data, gave the best discrimination of the samples and explained around 85% of the variance (Figure 3). This showed that ions with high abundance contributed strongly to the discrimination of the samples by the PCA.

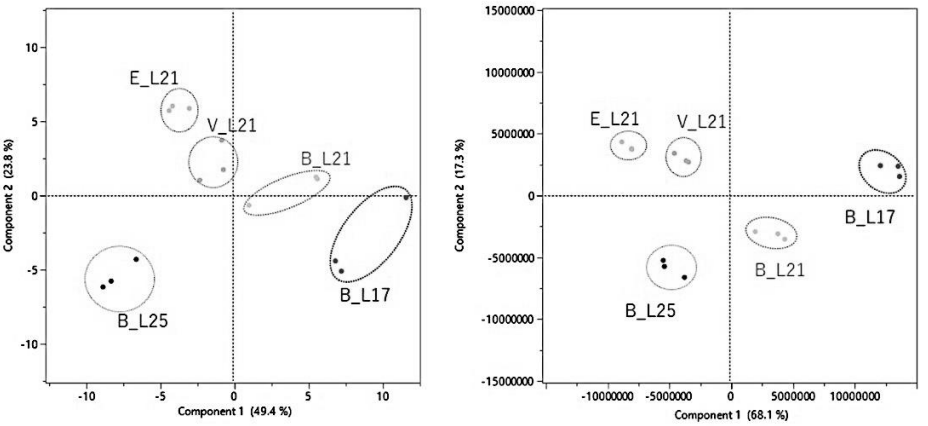

Figure 3: PCA plots of normalised data (left) and absolute data (right) using correlation and covariance matrices

Further analysis indicated that the same discrimination could be achieved with 19 high abundance ($>10^6$) ions and these represented the key drivers of the differences in the coffee headspace samples. Therefore, the pre-screen defined the next stage of analysis, which was to identify and quantify the nineteen compounds associated with the ions monitored in APCI using the GC-EI-APCI-MS technique [4].

Conclusion

Pre-screening of the coffee samples using APCI-MS and visualisation of the results, provided a rapid way to assess the differences between the samples. The information gained could be used to design subsequent analyses, in terms of chromatographic conditions and the type of internal standards needed. The concept is to get the GC- and/or LC-MS analyses right first time to avoid time delays due to having to re-run the analyses. APCI-MS provides an untargeted snapshot of the volatile profiles of the samples and, while not providing a comprehensive analysis, does cover off the main chemical classes involved (esters, acids, aldehydes, pyrazines etc.). Assessing the APCI-MS data in a visualisation package allows rapid data cleaning and checking, followed by easy-to-interpret plots that inform the design of the chromatographic procedures. The advantage of visualisation software is that any ion can be added to this chart using drag and drop, so the analyst can quickly test out ideas and hypotheses about the differences in the coffee profiles. Both JMP and Spotfire offer more sophisticated statistical analyses to interrogate the data. While statistical analysis is obviously key in identifying quantitative differences between samples, our experience is that data visualisation can complement the basic statistical analyses (e.g. ANOVA) and provide a focus for further chemical and statistical analyses.

References

1. Acree, T.E., Butts, R.M., Nelson, R.R., Lee, C.Y., (1976) Anal. Chem., 48: 1821-1822.
2. Grosch, W., (1993) Trends Food Sci. Technol., 4: 68-73.
3. Hatakeyama, J., Davidson, J.M., Kant, A., Koizumi, T., Hayakawa, F., Taylor, A.J., (2014) Food Chem., 157: 229-239.
4. Taylor, A.J., Sivasundaram, L.R., Linforth, R.S.T., Surawang, S., (2003) In: Handbook of flavor characterization. (Deibler, K.D., Delwiche, J., Eds.); Marcel Dekker: New York: p. 411-422.