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Classification of Food and Flavor Samples using a Chemical Sensor

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ABSTRACT
A mass spectrometry based chemical sensor consisting of a headspace autosampler directly coupled to a quadrupole mass spectrometer was used in three different food and flavor applications; strawberry flavors, whiskeys and soft drinks. This instrument integrates multivariate data analysis in which the mass spectra of the samples are used as fingerprints. Inconsistencies in raw materials were examined by analyzing flavors. Possible adulteration was studied by analysis of two whiskies. Multivariate models were able to detect whiskeys aged for different periods of time. Differences in similar product lines were studied using four soft drinks. Using this chemsensor differences were observed in one soft drink packaged in aluminum cans and plastic bottles.

INTRODUCTION

Identification of product adulteration, contamination or inconsistency in food and flavor samples requires short analysis times. Chemical sensors are ideal for these types of applications because they provide real-time results. While analysis times are crucial, accuracy of the analysis should never be compromised. It is therefore desirable to use a reliable and stable technology that is robust to environmental changes such as humidity or temperature [1]. Quadrupole mass spectrometry is a robust technique that has been widely used in food and flavor applications mostly coupled to a gas chromatograph.

In this study headspace sampling without chromatographic separation is performed using a quadrupole mass selective detector. The resulting composite mass spectrum of each sample is used to train the chemical sensor using multivariate pattern recognition techniques. Unknown samples are easily compared to standards using integrated software that can be easily customized to reflect pass or fail decisions.

In order to illustrate the potential of this technology, three different applications will be explored. For quality control analysis a series of strawberry flavors are examined [2]. Differences in these samples could reflect inconsistencies in raw materials important to a manufacturer of a more complex product such as yoghurt. For identification of adulteration, whiskeys aged different periods of times spiked with adulterants are investigated [3]. Differences in product lines were studied using soft drinks [4].

EXPERIMENTAL

Materials. Commercial strawberry flavors used were obtained from Zentis, Germany. A lesser value whiskey and whiskeys aged 4 and 10 years and soft drinks were purchased at a local store. Soft drink brands A and B were purchased in aluminum cans. Brand C was purchased in transparent plastic bottles (C-bottle) as well as aluminum cans (C-can).

Instrumentation. The chemsensor used was a Gerstel ChemSensor 4440 (Figure 1) that includes a headspace sampling unit (7694, Agilent Technologies) with a mass selective detector (5973N, Agilent Technologies). This instrument integrates chemometric software from Infometrix (Pirouette 3.02 and Instep 1.2). The instrument was used in the scan mode for the strawberry flavors (35-150 amu) with 1.5-min runs. With the six-sample overlap-heating feature of the autosampler oven, samples can be analyzed every 3 to 4 min. Therefore a tray of 44 samples can be analyzed in about 3 hours. The soft drinks were scanned from 46 to 150 amu with 0.75-min runs. Experiments for the whiskey samples were 1.00-min runs with a scan range of 48 to 170 amu.

Headspace sampling. 1-ml aliquots of each different strawberry flavor were placed into 10-mL vials, which were crimped and equilibrated for 15 minutes at 60 °C before headspace sampling. Since the GERSTEL ChemSensor 4440 does not use a column for a separation prior to the mass selective detector (MSD), the entire headspace of each sample is introduced into the MSD. 5-mL aliquots of soft drink samples and whiskeys were equilibrated 20 minutes at 80 °C and 75 °C respectively.

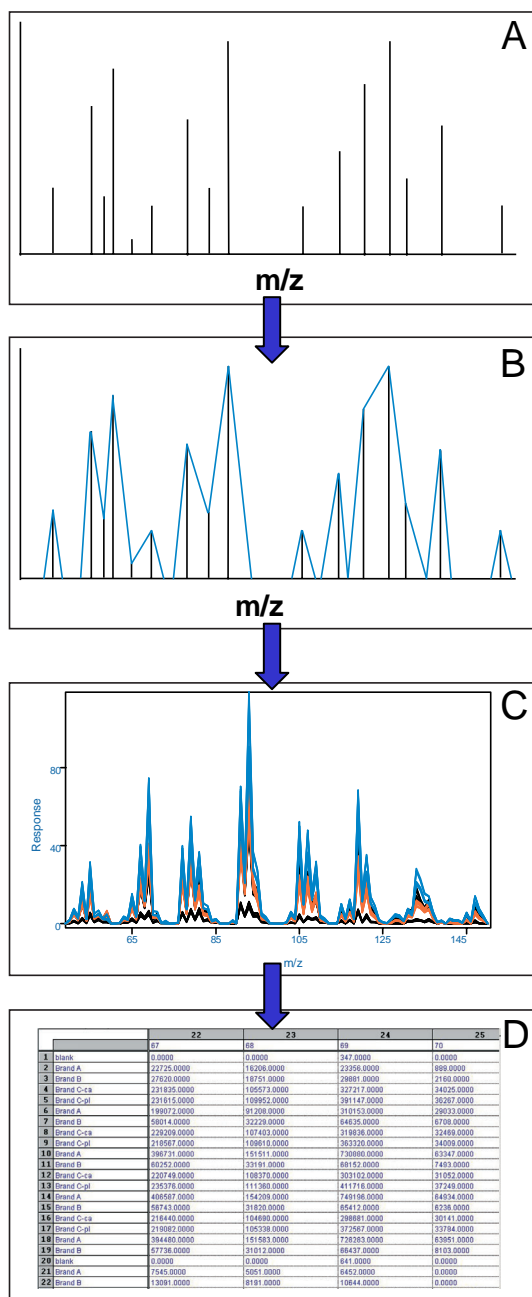
Figure 1. Gerstel ChemSensor 4440.

RESULTS AND DISCUSSION

Direct transfer of headspace volatiles using the GERSTEL ChemSensor 4440A results in fast analysis times. For example, Figure 2 shows the total ion chromatogram (TIC) obtained in 0.75 minutes for two soft drinks samples. Since there is no chromatographic separation, a single broad peak is normally obtained. The corresponding mass spectrum of each sample can then be used as a fingerprint. For example, Figure 2A shows the MS for the soft drink of Brand C from a plastic bottle. Comparison to the MS obtained for Brand C in the aluminum can (Figure 2B) indicates differences in the abundances of some ions such as 46, 69, 93, 119, etc.
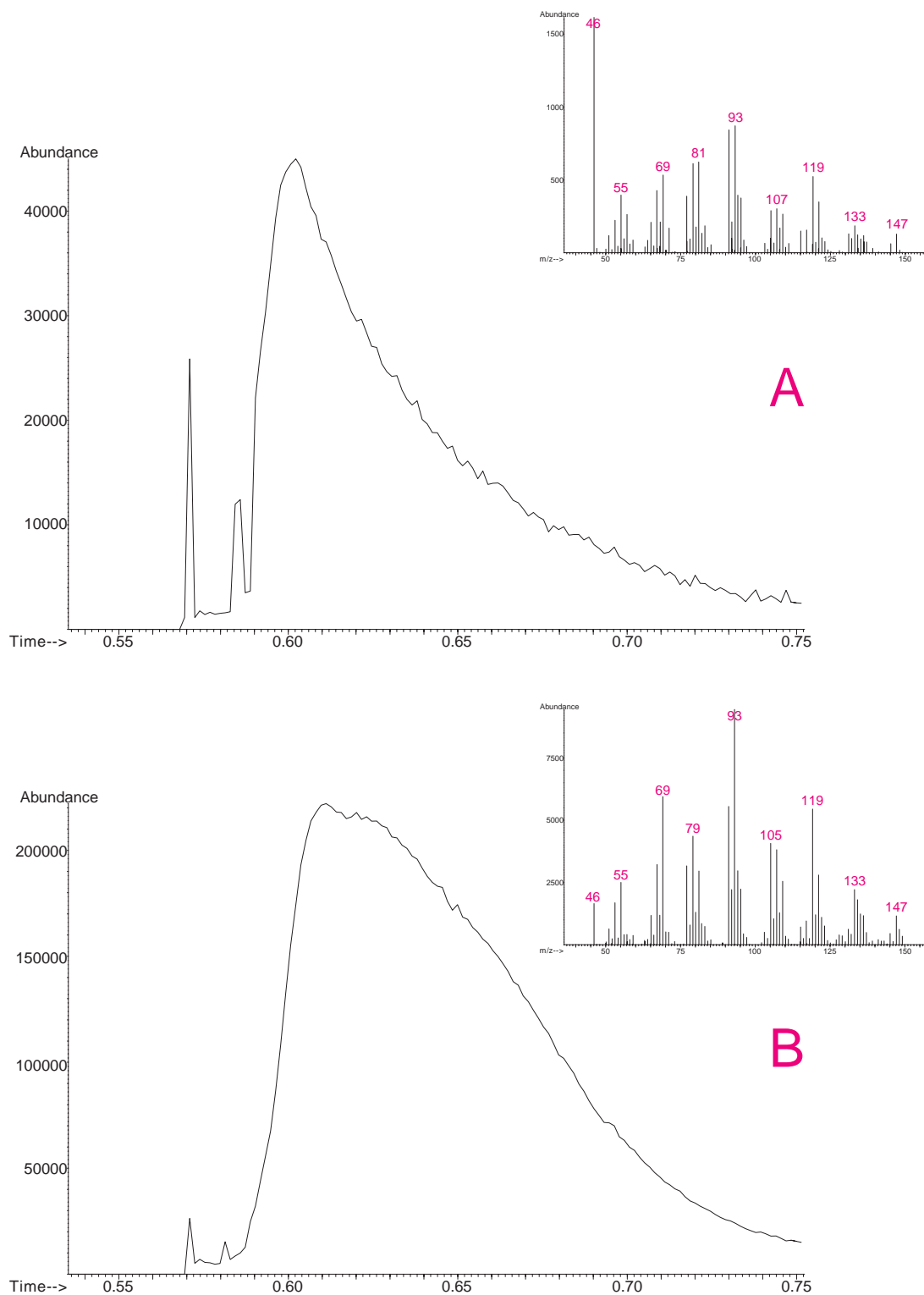
Figure 2. TIC and MS for brand C of soft drink. (A) in plastic bottle and (B) in aluminum can obtained with Gerstel ChemSensor 4440.

The mass spectrum obtained for each sample can also be represented as a line plot (Figure 3). Customized macros, especially designed for the GERSTEL Chem-Sensor 4440A, create an ASCII file for each sample and a global, composite matrix for each sequence. Chemometrics data analysis is then performed on the composite matrix that contains the mass spectra of the samples. As seen in Figure 3, the line plot data can visualize differences between samples as in ion abundances or the presence or absence of certain masses.

Figure 4 illustrates the four basic steps necessary to use the GERSTEL ChemSensor 4440. During the training mode, the headspace of standard samples is introduced into the MSD. The mass spectra of these standards become like "fingerprints" for future unknown comparisons. In the second step, multivariate models are created that take into account all the masses collected in the scan range set by the operator. In the prediction mode, unknown samples are compared to the chemometric model. Last, final answers are obtained for unknown samples that can easily be interpreted by line operators.

Figure 3. Mass spectra from standard (A) produces a line plot (B) that can be overlaid with other samples (C). Special macros create the ASCII file (D) for each sample and compile each sequence into a global data matrix.
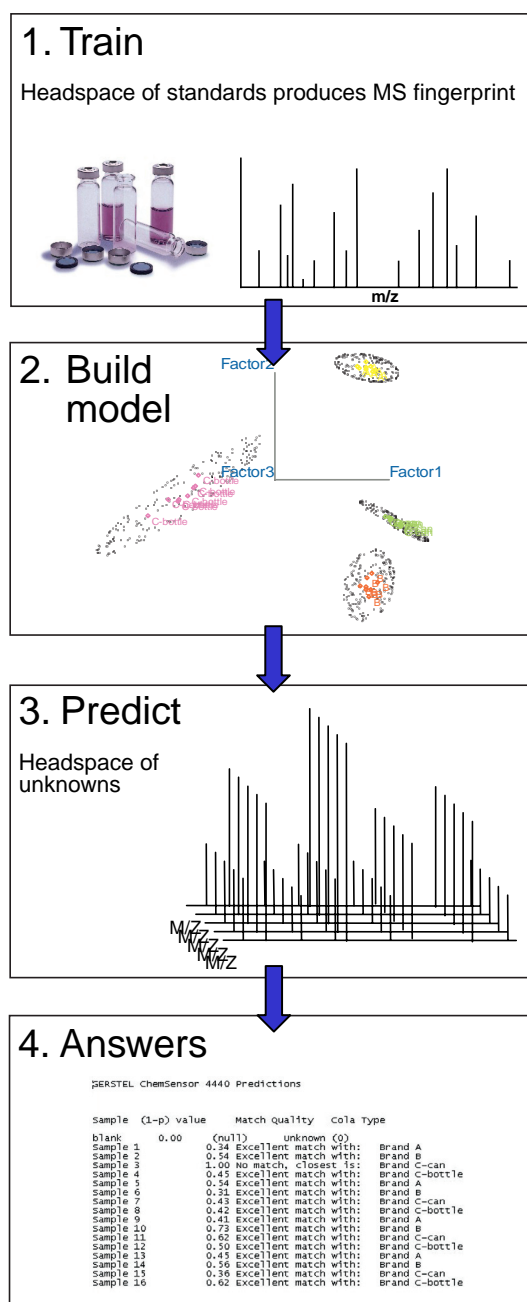
Figure 4. Steps used to obtain answers using the GERSTEL ChemSensor 4440.

Reliable chemometric models include only standards representative of acceptable samples. Since random and systematic errors are normally part of every measurement, the raw data needs to be closely examined. Assuring the validity of the raw data is accomplished using exploratory algorithms, such as hierarchical cluster analysis (HCA) and principal component analysis (PCA). The goal of exploratory data analysis is to detect unusual samples (outliers) and to detect natural groupings in the data set. For example, Figure 5 shows the dendrogram obtained using HCA on the bourbon samples using Euclidean distance and incremental linkage.
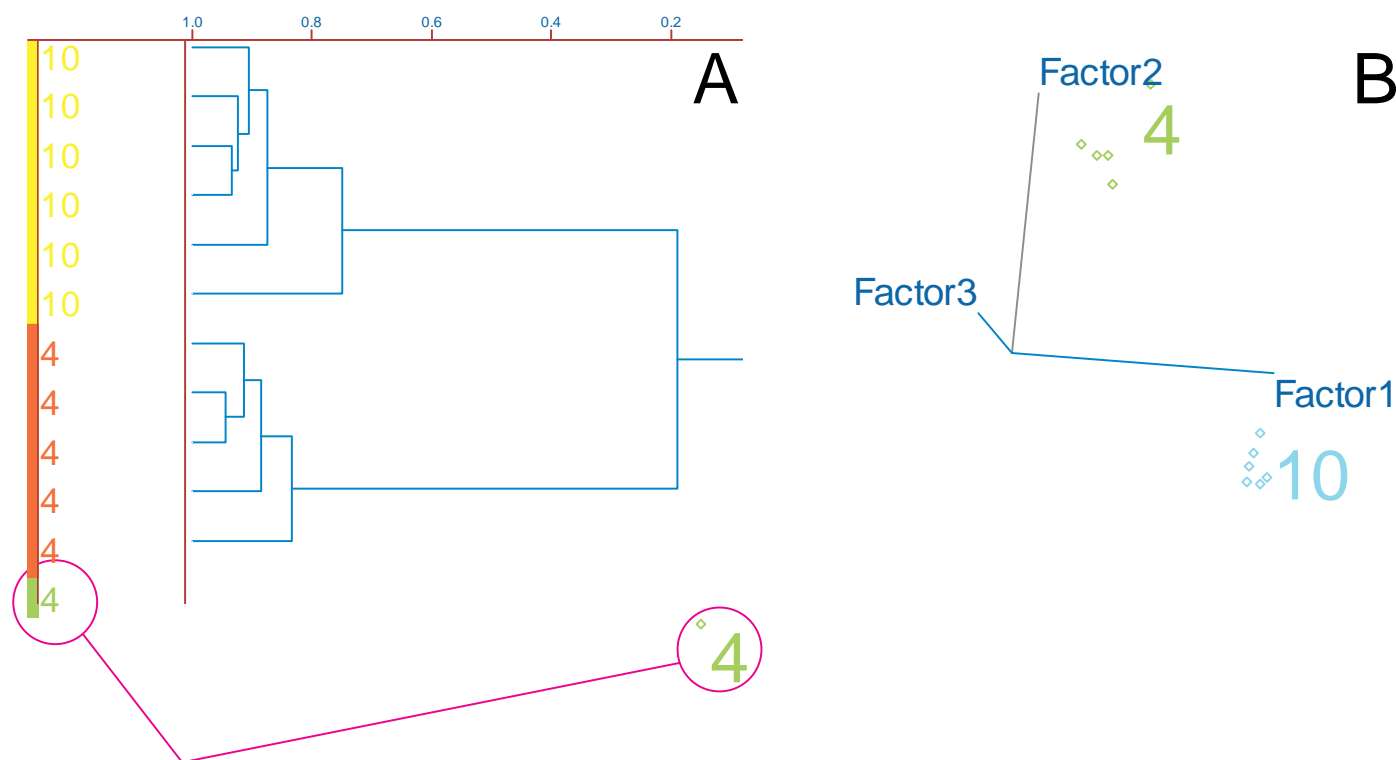
Figure 5. Exploratory analysis of whiskeys samples. A) Hierarchical cluster analysis using Euclidean distance and incremental linkage. B) Projections of the mass spectra of whiskeys samples into the space of the first three principal components.

Two clear clusters are formed but also an unusual sample from bourbon aged 4 years can be seen in the lower part of the dendrogram. A scores plot obtained using PCA on the same data set indicates the same unusual sample. A reliable model must exclude this unusual sample from any chemometric model.

Once the raw data has been validated, classification or regression models can be built. The GERSTEL Chem-Sensor 4440A offers two classification algorithms: soft independent modeling of class analogy (SIMCA) and K-nearest neighbors (KNN). Regression models include principal component regression (PCR), partial least squares (PLS) and classical least squares (CLS).

An example of a class projection plot for a SIMCA model is shown in Figure 6.
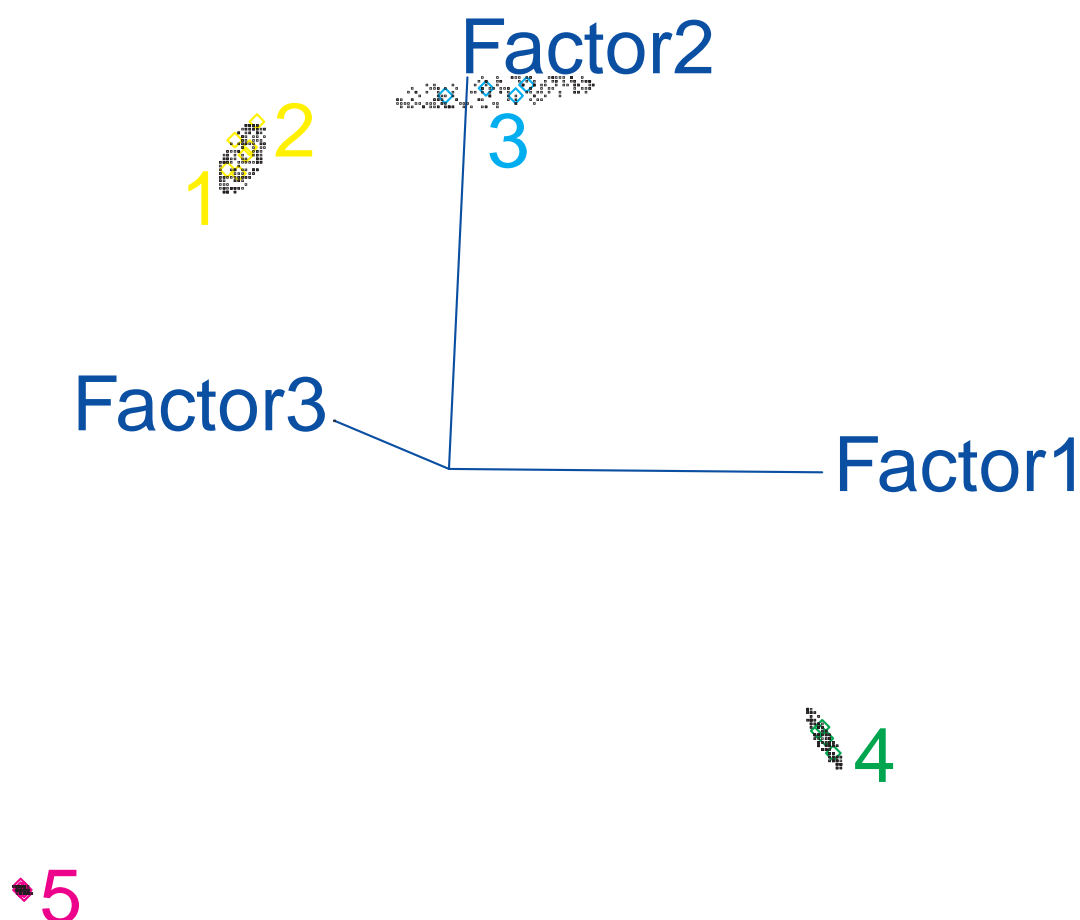
Figure 6. Projection of the flavors mass spectra into the space of the first three principal components. 1&2=strawberry, 3=raspberry, 4= pear and 5= passion fruit.

For this type of analysis four different commercial flavors were collected from different suppliers. Inconsistencies in the same type of flavor from different suppliers were detected using a classification model. SIMCA develops principal component models for each category of the training set. The bounding ellipses form a 95% confidence interval for the distribution of these categories. In this case, the projection of the mass spectra of the four flavors indicates good clustering between samples without overlap. Another indication of a good SIMCA model is the interclass distances between samples (Table 1).

Table 1. SIMCA interclass distances.

	CS1&2@2	CS3@2	CS4@1	CS5@2
CS1&2	0.00	10.02	94.02	81.38
CS3	10.02	0.00	81.49	72.54
CS4	94.02	81.49	0.00	197.07
CS5	81.38	72.54	197.07	0.00

This measurement indicates how well the classes are separated from each other. As a good rule of thumb, interclass distances greater than 3 are considered well separated. For the flavor samples these distances indicate good separation between samples.

A PLS regression model was also created for flavor samples.

Figure 7 shows the prediction vs. the known concentration. Zero stands for pure strawberry and 1000 for pure raspberry flavor (Table 2). The two samples annotated 350 are classified as pure raspberry flavors (Table 3). For these samples addition of strawberry flavor (650 µL) was accidentally forgotten. Slight discrepancies in the prediction of the 210 µL samples suggest slight error in their preparation.
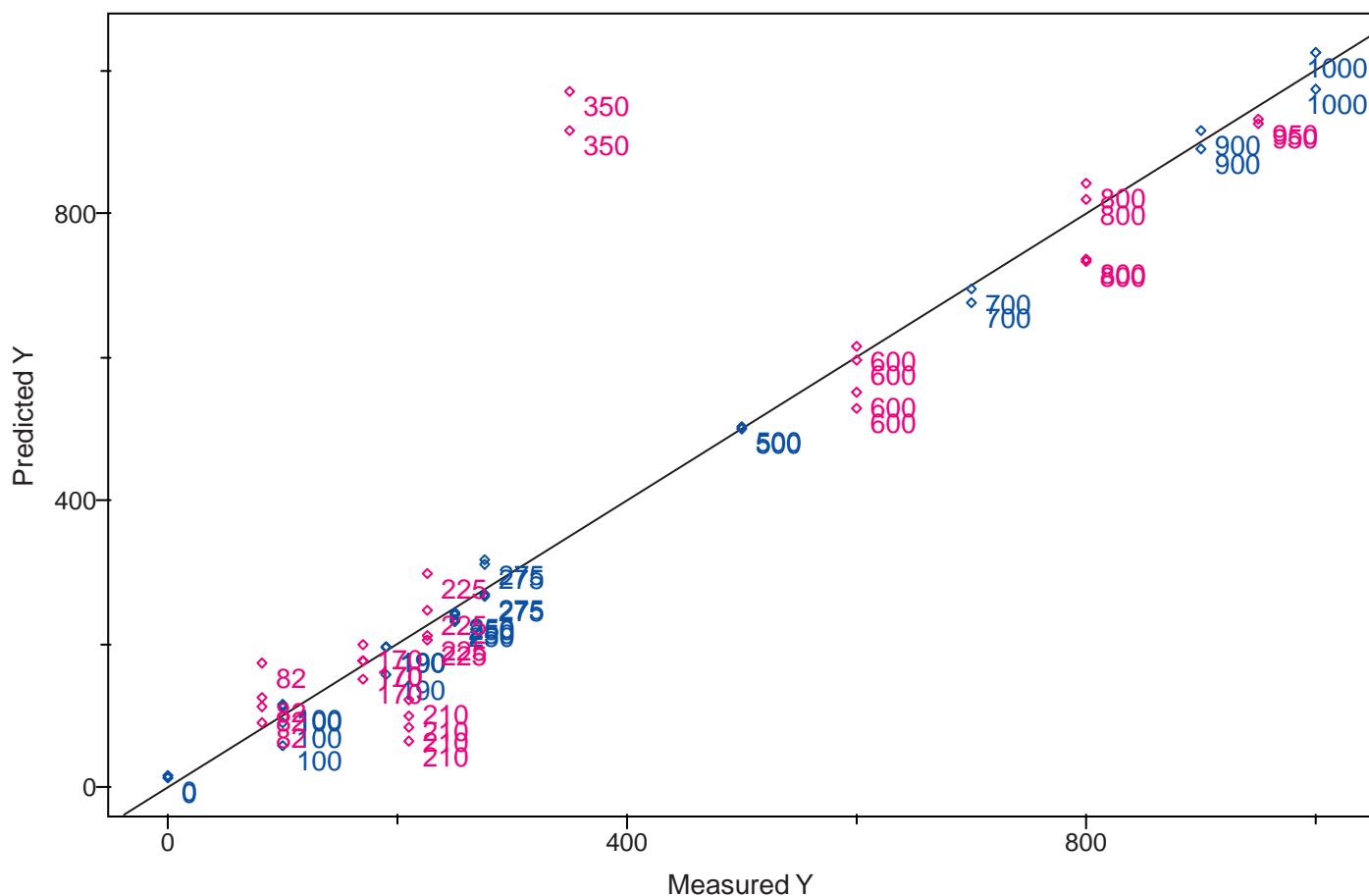
Figure 7. Prediction (red) of strawberry/raspberry flavor mixtures vs. a PLS model (blue).

Table 2. Ratio of strawberry and raspberry flavors used to create a PLS model.

Raspberry [µL]	Strawberry [µL]
0	1000
100	900
190	810
250	750
275	725
500	500
700	300
900	100
1000	0

Table 3. Ratio of strawberry and raspberry flavors used to predict against the PLS model.

Raspberry [µL]	Strawberry [µL]
82	918
170	830
210	790
350	0
600	400
800	200
950	50

Detection of adulterated bourbons is shown in Figure 8. In this plot, the mass spectra of spiked bourbons were projected into the space of the standard samples (Figure 5). The ellipses in the plot do not represent statistical information and are provided for visual identification of clusters only. It is clear that a PCA plot can easily detect differences in the mass spectra of the adulterated samples.
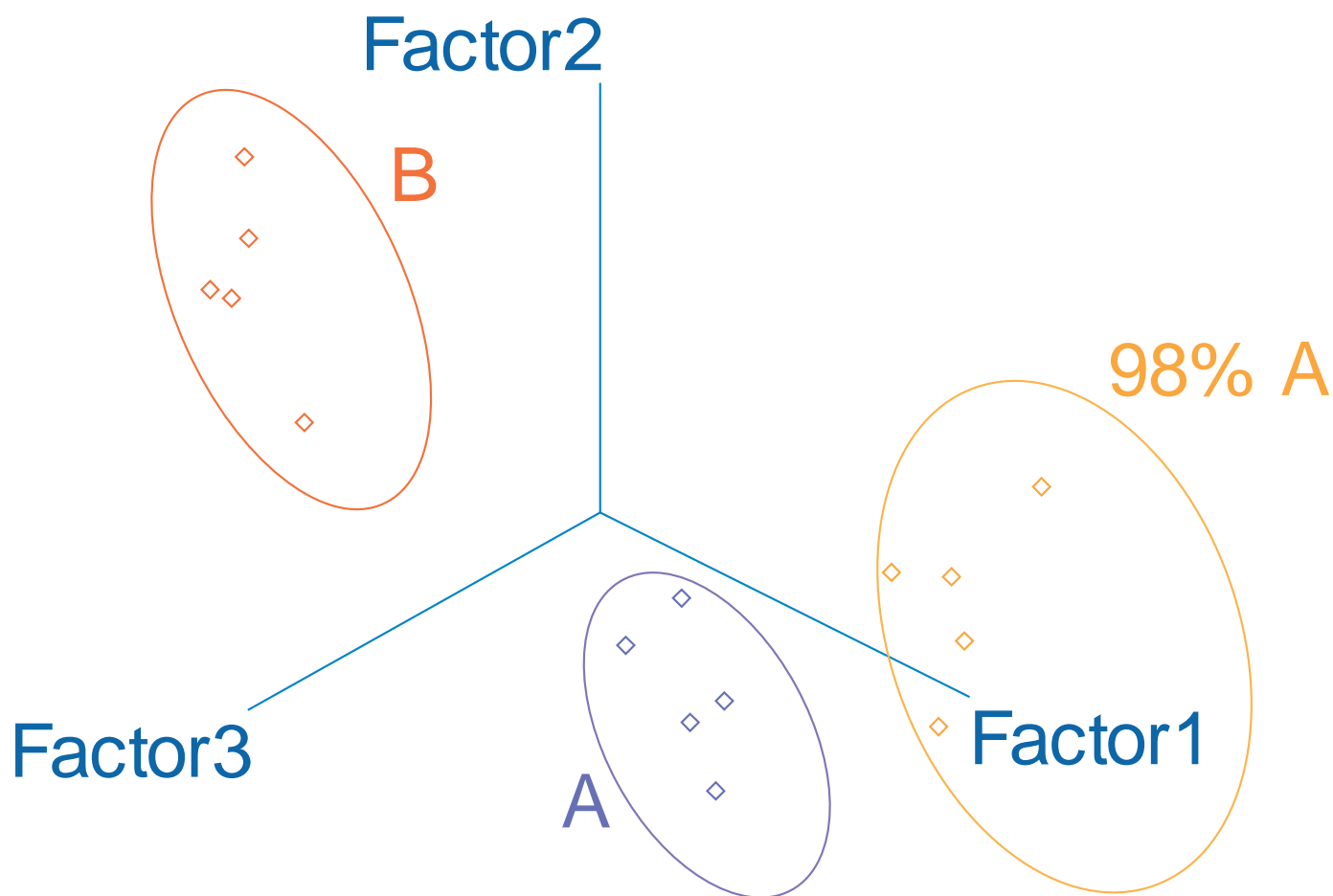

Figure 8. Projection of 98% Bourbon A with 2% Bourbon B in the space of the first three principal compon-

Projection of the mass spectra of the four soft drinks into the space of three and two (Figure 9) principal components shows good clustering between replicas. Since over 90% of the variance was captured within the first 3 PCs, we can be confident that differences in the samples scores are differences in the soft drinks headspace. The first PC (horizontal axis in Figure 9B) explains the difference between brand C in the plastic bottle and the rest of the samples. This indicates that the headspace of brand C in the bottle is very different than the headspace from the other sodas. The second PC (vertical axis of Figure 9B) indicates differences between brands B to A and to C-can.
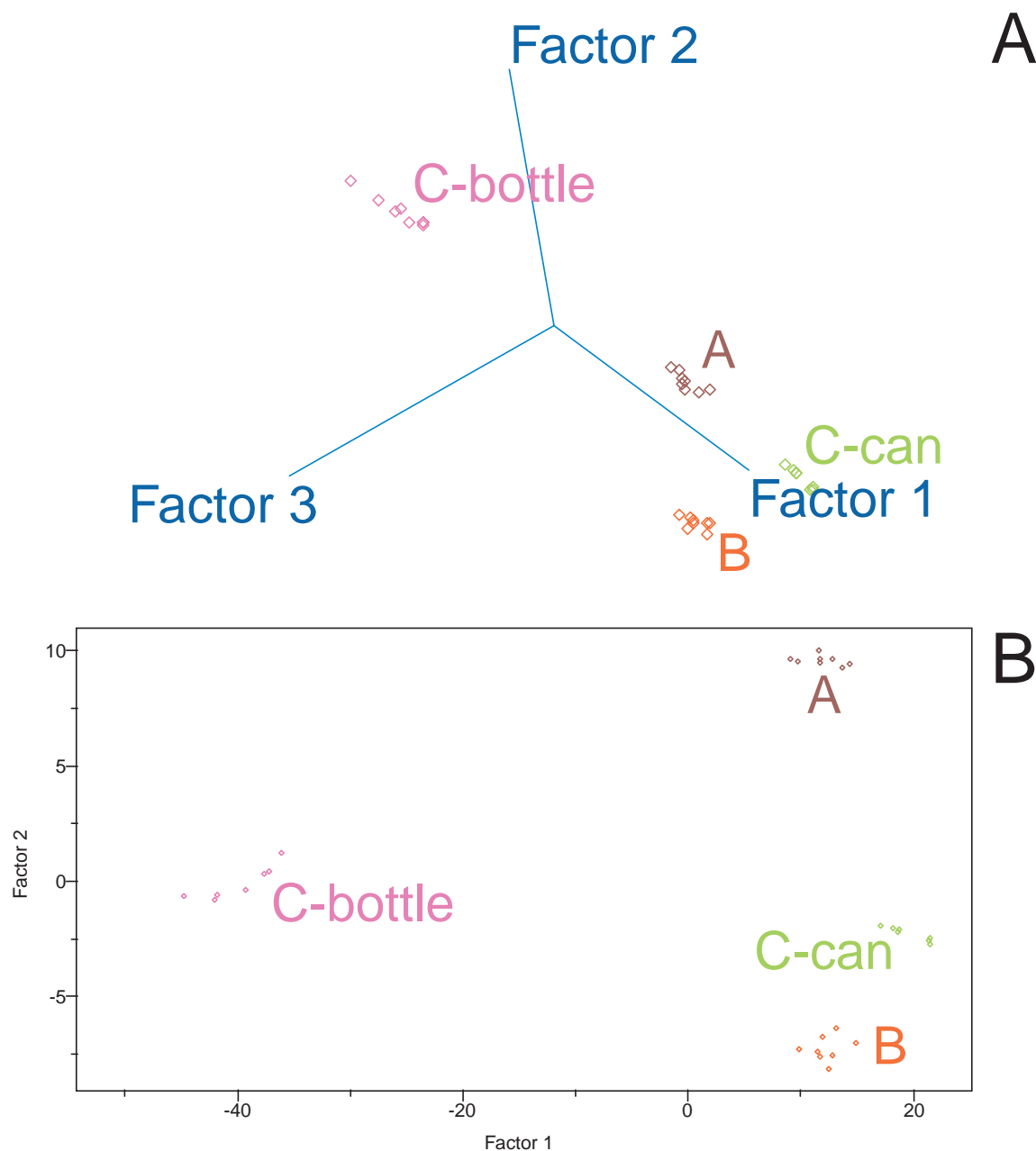
Figure 9. Projection of the sodas mass spectra into the space of the first three (A) and two (B) principal components.

CONCLUSION

The fast and accurate classification of samples using an instrument that integrates multivariate statistics with mass spectrometry technology is now possible. The GERSTEL ChemSensor 4440A has proven to be capable of detecting differences in the quality of incoming flavors.

Using PCA adulterated bourbons were detected in the low percentage range as well as differences in the chemical composition of soft drinks headspace. These results are also in agreement with cluster analysis.

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