Apple flavors and essences are food ingredients that emit a vast array of aromatic volatile gases, which contribute to the sensorial quality of foods in which they are incorporated (MacGregor et al., 1964). The headspace atmosphere of different flavors and essences are distinct both qualitatively and quantitatively (Morton and MacLeod, 1990; Dimick and Hoskin, 1983). Reliable, inexpensive analytical methods to accurately detect and differentiate flavors and essences are needed. The human nose as a sensory analytical tool remains the most widely used technique for analysis of flavors and essences. Early instrumental detectors, categorized at that time as electronic noses or e-noses, utilized a traditional analytical approach of spectroscopy or chromatography. The headspace atmospheres of flavors and essences were evaluated with either spectral or temporal separation, and also evaluated by the human nose after separation of components. Newer detectors rely on chemometrics, and vary from solid-state sensors to rapid-analysis mass spectrometers (Sarig and Delwiche, 1998). Disadvantages of solid-state sensors include sensor poisoning, no chemical structure information, time-consuming calibration, short- and long-term drift, and water and alcohol interference (InfoMetrix, 1999). An array-type chemical sensor designed to alleviate these disadvantages was developed by Cyrano Sciences (Doleman et al., 1998). This sensor array is based on the physical characteristic that carbon black organic polymer composites swell reversibly upon exposure to volatile compounds. This sensor array is fast when compared to the response times of gas chromatographs and mass spectrometers. Recent studies compare the pros and cons of gas chromatography and mass spectroscopy (Heiden et al., 2002) to measure aromatic volatile headspace gases. The need exists to compare a chemical sensor array to standard analytical instruments. The chemical sensor array’s quick response time, instrument portability and ease of use would facilitate industrial and laboratory applications to characterize the aromatic volatile gases in the headspace atmosphere of foods and food ingredients.

The flavor and essence industry is a significant part of the food industry. Among their product line is a vast array of apple flavors (naturally occurring apple components and other flavor-associated components) and essences (apple concentrates). These products, which contain aromatic volatile compounds, are combined and incorporated into a variety of processed foods to impart aromas and flavors reminiscent of apples. The primary objective of this study was to determine the feasibility of comparing the prototype...
Cyrano Sciences chemical sensor analyses of apple flavors and essences to standard analytical approaches for matching and differentiating different apple flavors and essences. Headspace atmospheres of known and unknown flavors and essences were measured with a prototype Cyrano Sciences chemical sensor and with a gas chromatograph.

**MATERIALS AND METHODS**

**FLAVORS AND ESSENCES**

Twenty apple flavors (generous gifts from Alex Fries, Cincinnati, Ohio; McCormick, Hunt Valley, Md.; and Takasago, Rockleigh, N.J.) and four apple essences (generous gifts from Flavor Materials International, Avenel, N.J.; and Sun Pure - Mastertaste, Lakeland, Fla.) were stored in the dark at 4°C between uses. After each use, the headspace of each storage bottle was flushed with nitrogen gas and resealed. While these efforts were made to maintain product stability, some product degradation may have occurred during the course of this study. Formulations for these samples are proprietary. Notation referring to specific products was replaced with code numbers 1 through 24. In addition, to serve as a repeat “blind” measurement, eight of the same products (six flavors and two essences) were randomly selected and coded with the numbers U1 through U8. To avoid bias, the identity of the blind products was not revealed until after all of the sample headspace atmospheres from the coded flavors and essences had been measured and the data analyzed.

**SAMPLE IDENTITY**

After all headspace volatile measurements were completed and the data analyzed, the identities of the coded samples were revealed. Two of the coded samples were essences, and the other six were flavors.

**GAS CHROMATOGRAPHIC ANALYSES**

Aliquots (0.5 mL) of each coded product were transferred to individual 10 mL vials and crimp capped with a Teflon-lined septum. The samples were equilibrated for 5 min at 20°C. For headspace volatile sampling, a solid-phase microextraction (SPME) fiber coated with polydimethylsiloxane (PDMS, 1 cm long, 100 µm thick, Supelco Co., Bellefonte, Pa.) was used to collect and concentrate aromatic volatile gases in the headspace of the sample vials. These fibers act by virtue of their sorption characteristics (Arthur et al., 1992). After 16 min of exposure time, the sorbed volatile headspace gases were desorbed from the fiber for 2 min at 250°C (injector port temperature) into a glass-lined splitless injection port of a gas chromatograph (model 5890a, Agilent Technologies, Rockville, Md.) equipped with electronic pressure control and a flame ionization detector (FID).

Apple flavor and essence volatile headspace gases were separated using a capillary column (HP-5, 11 m × 0.1 mm ID, 0.34 µm coating thickness). The carrier gas was ultra-purified hydrogen (6.0 research) at a flow velocity of 52 cm s⁻¹. The temperature program was isothermal for 2 min at 40°C and then raised at a rate of 30°C min⁻¹ to 250°C and held for 3 min. For identification of unknown samples, chromatograms of each unknown sample (coded U1 to U8) were visually compared to chromatograms of each known sample (coded 1 to 24). This visual examination and the chemometric analysis used for the chemical sensor were the methods of choice of the GC expert and statisticians consulted for the project.

**CYRANO SCIENCES MULTIPLE ARRAY CHEMICAL SENSOR - DATA LOGGER prototype**

The multiple array chemical sensor - data logger used in this study was provided by Cyrano Sciences, Inc., Pasadena, Cal. (prototype data logger serial number DL-0027-012999, sensor set number CN-7100A-608). It is a portable polymeric resistance change sensor unit that connects to a power supply and a laptop computer through a parallel printer port. The instrument is sometimes referred to as a data logger, a multiple array chemical sensor, a chemical sensor, an “e-nose,” or an “electronic nose.” The chemical sensors (32 total) are carbon elements impregnated with non-conducting polymers whose resistance changes when exposed to different volatile headspace gases. Only 31 of the 32 sensors were used in this study since the manufacturer indicated that the output from one of the sensors was unreliable. The custom-made chemical sensors in the prototype data logger used in this study are not expected to be as stable, accurate, or precise as those manufactured using refined mass production techniques. In this regard, subsequent performance by production versions of the portable chemical sensor unit should improve due to standardized sensor manufacturing methods.

**CHEMICAL SENSOR ANALYSES**

A one-half mL aliquot (0.5 mL) of each flavor and essence was transferred to a 500 mL Erlenmeyer flask and sealed with a Teflon-lined rubber stopper. Samples were allowed to equilibrate for 5 min at room temperature (23°C ±1°C). Cyrano Sciences designated the sampling protocol cycle as follows: a 10 s baseline, room air flush, a 25 s headspace sampling, and then a 60 s, room air flush. Sensor chamber temperature was maintained at 28.0°C, and flow rate through the sensor chamber was kept at 150 mL min⁻¹ using the default pump setting (3 V). A single data set consisted of the average of five consecutive samplings of the same sample. Since immediate data analysis was not possible, two data sets were consecutively collected. Only data sets in which the headspace atmosphere maintained an apparent steady state throughout sampling were used for analysis. The data were normalized for each of the sensors to equalize the concentration effect.

**RESULTS AND DISCUSSION**

**GAS CHROMATOGRAPHY**

Composite chromatograms of representative flavors and essences are shown in figure 1. These matches were made by visual analysis of gas chromatograms, and by analytical evaluations (PCA and HCA) of data sets from the chemical sensors of the prototype data logger and their coded identification. The chromatograms from many of the 26 flavors (20 known and six unknown coded samples) and six essences (four known and two unknown coded samples) were unexpectedly similar to each other in peak profiles. As expected, the chromatograms of the essences generally had higher peak magnitudes than that of the flavors. However, there were sufficient qualitative and quantitative differences...
among the chromatograms of the flavor samples to find absolute matches for five of the six unknown flavors by visual inspection (table 1). The chromatograms of the two unknown essences were too similar to one another and to several of the known flavor samples to correctly identify, even though many of the known flavor samples could be excluded due to their relatively low volatile abundance. From the gas chromatographs, four of the flavors had high levels of ethanol, which may have been added as a flavor enhancer (data not shown). This segregated those samples from those without high levels of ethanol and increased the chance of obtaining a correct unknown sample identification.

**Cyrano Sciences Chemical Sensor Measurements Statistical Analysis**

Principal component analysis (PCA), multivariate hierarchical cluster analysis (HCA) and pattern recognition analyses were run with SAS software (SAS, 1997). These analyses were used to discriminate among various samples. The mean of five volatile samplings for each flavor, essence, or unknown coded sample was used for data analysis. The data sets from 31 sensors of the data logger were analyzed using PCA. The PCA successfully reduced the data sets from the sensors to two principal factors, which accounted for 96.4% of the variance in the sensor measurements. The data from the two principal factors were analyzed using HCA.

The results of the HCA analysis using average linkage for cluster formation suggest two clusters. The cluster output is shown in figure 2. The first of the clusters contains flavor 19. The other cluster is split into two subclusters: flavors 5 and 14, and all remaining coded flavors and essences. Within each designated cluster, HCA indicated sample similarity groupings that were used to match and differentiate unknown coded samples to known coded samples.

The Cyrano Sciences chemical sensor correctly identified all six unknown flavors. Three were absolute matches, and three matched more than one unknown (one of which was the correct flavor). The two unknown essences were not identified correctly.

**Comparison of Unknown Determinations**

Visual comparison of gas chromatograms and HCA sample groupings was used to identify and discriminate unknown and known coded samples and source bottle designations (table 1). Table 1 also lists the sample type, either flavor or essence. A comparison of the results showed that in sample set 1 (U1, U2, U3, and U4), the gas chromatographic and the Cyrano Sciences chemical sensor data analyses correctly matched the three unknown flavors (U1, U2, and U4). The analysis of the chemical sensor data for U2 did not exclude incorrect sample 16. Sample set 2 (U5, U6, U7, and U8) was measured after an additional one-month storage period and may have undergone some storage-related degradation before measurement. For set 2, the chemical sensor analyses correctly identified the three unknown flavors (U5, U6, and U8) but did not exclude incorrect matches for samples U6 and U8. The gas chromatographic technique led to two correct unknown flavor matches (U5 and U6) and one incorrect match (U8). Neither analytical technique correctly identified the unknown essences (U3 and U7). Both techniques incorrectly identified the unknown essences as flavors having relatively high total volatile abundances.

These results indicate that the chemical sensor’s capability to perform this kind of separation shows promise but is not consistent (two absolute matches for sample set 1, and only one absolute match in sample set 2). This compares to three absolute matches in set 1 and two in set 2 for the GC. The accuracy and discriminatory ability of the chemical sensor technique would be improved by identifying those sensors or sensor combinations that respond differentially to flavor- and essence-related aromatic volatile headspace gases and are little affected by interfering gases such as CO₂ and H₂O.
Figure 2. Cyrano Sciences chemical sensor HCA cluster analysis of flavors and essence samples 1 to 24 and eight unknowns (U1 to U8). The HCA input was the first two principal factor results from a PCA analysis that includes 96.4% of the variance. All sensors except 31 were used in the analysis.

Improved performance of chemical sensors may also occur following detailed statistical analyses of data sets from different sensors or sensor sets (Marrazzo, 1999). In addition, alternate types of classification techniques such as neural networks (that are trained with known samples in order to classify unknowns) may provide better results.

CONCLUSIONS

Data sets from 31 sensors of the data logger were analyzed using PCA. The PCA analysis of the data sets identified two principal factors. Further analysis of the chemical sensor data sets using HCA with average cluster formation indicated that 22 of the 24 flavors and essences clustered together and thus could not be distinguished from one another. The same analysis provided a breakdown of the clusters into subgroups by similarity. These were used to determine "matches" with six unknown flavor and two unknown essence samples. The chemical sensor and clustering technique correctly identified all six unknown flavors (three were absolute matches). By comparison, a gas chromatographic technique was less ambiguous (all matches were absolute) but also less accurate (five unknowns were correctly identified) at identifying the unknown flavor samples. Neither technique identified the unknown essences.

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