A REFEREED PAPER

DIFFERENTIATING ORANGE JUICES USING FOURIER TRANSFORM INFRARED SPECTROSCOPY (FT-IR)

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Additional index words. multivariate, statistics, PCA, DFA, ANOVA. MS, GC

Abstract. **Fourier transform infrared spectroscopy (FT-IR) is compared to various instrumental and physical/chemical methods for differentiating commercial orange juice products. Previous statistical models were generated using the data from an electronic nose (e-nose), a head space gas chromatograph (GC), a mass spectrometer (MS) based chemical sensor, and various physical/chemical measurements. The separation using data from the FT-IR was similar to that obtained from the instrumental methods (e-nose, GC, MS) and chemical/physical measurements. FT-IR is inexpensive technology and common in most labs. An advantage to using an FT-IR would be the likelihood of quality labs currently owning an instrument, or if not, the relatively low cost would not be prohibitive. Seven not-from-concentrate and 3 from-concentrate orange juice products were analyzed with excellent separation using the data from standard procedures. This compares favorably with the other methods examined in previous years.**

The electronic nose is an important QA/QC tool used in many industries. Typically these instruments utilize advanced multivariate statistics coupled with a non-specific chemical sensor array in order to differentiate samples (Goodner et al., 2000). However, any type of sensor that responds to chemicals can be used as a replacement for the electronic nose. For example, a flame ionization detector (FID) for a gas chromatograph (GC) can be used for a chemical sensor. As each compound elutes from the GC, the FID produces a response. The individual peaks of the chromatogram become the 'sensors' with the advantage that the peaks are likely single compounds and thus the model can be related to specific chemicals. This method is different from a traditional electronic nose since there is chemical separation of the individual constituents. There are currently no commercially available GC-FID electronic nose instruments.

In the case of a mass spectrometer (MS) based electronic nose, each mass to charge (m/z) is used as a 'sensor' (Goodner et al., 2002). There is no chemical separation of the sample prior to analysis, so the mass spectra are representative of the entire product. This lack of chemical separation is similar to

the typical electronic nose method of introducing the sample to a non-specific sensor array, which does not give individual chemical separation. Some of the advantages of the MS are apparent: sensitivity, selectivity, number of sensors, speed, and some basic information on mass range can be determined.

Any type of measurement can be used as a sensor, not just instrumental data. Traditional citrus processing utilizes many standard tests, some of which are decades old, but could be used as "sensors." Citrus processors have a tremendous amount of data available to them from the many analyses they have performed over the years. The most common tests are for °Brix (soluble solids), titratable acidity, °Brix/acid ratio, pH, color, pulp content, Scott oil, and vitamin C (Goodner, 2004). Using these standard tests, a multivariate model can be built to differentiate orange juices.

An instrument found in many laboratories is the Fourier transform infrared (FT-IR) spectrophotometer. Infrared energy is absorbed by chemical bonds via vibrational modes such as bending, stretching, and twisting. The wavelengths of responses are indicative of certain types of chemical bonds (Silverstein et al., 1991). Similar to MS, each wave number is used as one of very large number of potential sensors. FT-IR and NIR (near infrared) have previously been used with multivariate statistics for some citrus products such as oils (Steuer et al., 2001), sugars and acids (Li et al., 1996), and adulteration issues (Kemsley et al., 1994).

Electronic nose instruments have been applied to citrus problems in the recent past (Bazemore et al., 1997; Goodner and Rouseff, 2001; Goodner et al., 2000, 2001a; Shaw et al., 2000). This report is a continuation of research reported previously describing the various abilities of electronic nose types to differentiate orange juice samples. This current multivariate model, based on FT-IR spectra of orange juice is compared to a traditional sensor-based electronic nose, a GC-based electronic nose, a MS-based electronic nose/chemical sensor, and to standard citrus processing analyses.

Materials and Methods

An Alpha Mos Fox 4000 e-nose (18 sensors) was used for all metal oxide sensor data collection. The specifics for the sample information, data collection, and analysis have previously been reported (Goodner et al., 2000).

The HS-GC (headspace gas chromatograph) system consisted of a Chrompak purge and trap headspace analyzer connected to an HP 5890 GC with an FID detector. The specifics for the sample information, data collection, and analysis have previously been reported (Goodner et al., 2001a).

The MS-based chemsensor used was a Gerstel ChemSensor 4440A that includes a headspace sampling unit (7694, Agilent Technologies, Palo Alto, Calif.) with a mass selective detector (5973, Agilent Technologies, Palo Alto, Calif.) for using each m/z as a sensor. The juice samples used with the chemsensor were not the same as with the electronic nose

and HS-GC. The specifics of the sample information, data collection, and analysis have previously been reported (Goodner et al., 2002).

°Brix and optical density were measured using an AR200 digital refractometer (Reichert, Depew, N.Y.). Titratable acidity (pH 8.2 endpoint) and pH were measured using a 614 Impulsomat, 605 pH-meter, and a 665 Dosimat (Metrohm, Herisau, Switzerland). Color was measured using a CR-300 Choma Meter (Minolta, Tokyo, Japan) (Anonymous, 1981). Vitamin C, Scott oil (Scott, 1966), and suspended pulp were analyzed using standard methods (Anonymous, 1981). The specifics of the sample information, data collection, and analysis have previously been reported (Goodner et al., 2004).

Ten orange juice samples were obtained from a local grocer. Seven were 'not-from-concentrate' and three were a 'from-concentrate' juice. Six samples were in gable top cartons and four in plastic containers (either polyethylene terephthalate [PET] or polyethylene). A Spectrum One FT-IR (Perkin-Elmer, Wellesley, Mass.) with the horizontal attenuated total reflectance (HATR) option using a 45° ZeSE trough plate was used to collect data. Spectra were acquired from 3000-900 cm^{-1} with a 0.25 cm^{-1} resolution with each wavenumber being a "sensor." Ten replications per juice (same juices used for the physical data experiments) were sampled. Data were analyzed with Unscrambler version 9 (Camo, Woodbridge, N.J.) and Statistica version 7. Discriminant function analysis in Statistica was performed using the first 10 principal components of PCA (Principal components analysis) transformed data.

Results and Discussion

Briefly, two types of statistical analyses were used for these projects—principal components analysis (PCA) and discriminant function analysis (DFA). These two analyses are similar, but with distinct differences. A PCA is a transformation of many variables into a linear combination of variables that maximizes the variance of the data into each of the principal components. This transformation is performed without regard to the classification of the samples. A DFA performs a similar transformation, but instead of maximizing the variance in each new variable, maximizes the distance between centers of defined classes.

The metal oxide based electronic nose provided adequate separation of the components as can be seen in Figure 1. This graph was produced by using a discriminant function analysis (DFA) with 15 variables (each coming from a single MOS sensory), which gave a data point to variable ratio of 3:1. There were three samples completely isolated from the others as the 90% confidence ellipses show with the FCOJ group being most differentiated. This is the best result obtained by Goodner et al. (2000) for the electronic nose.

Figure 2 is a graph of the DFA of the data from the HS-GC using a 3:1 data point to variable ratio (13 variables each representing a single chromatographic peak). One can see that there are four distinct groups: two that are composed of single samples and two that are composed of multiple samples. The ellipses drawn are for the 90% confidence interval. Once again, the FCOJ sample is well separated from the other samples and NFC 5 is well separated from NFC 2 and 4. The samples that have significant overlap have no obvious similarities (i.e., they are not multiple brands from the same producer, but it is possible that some are the same product under differ-
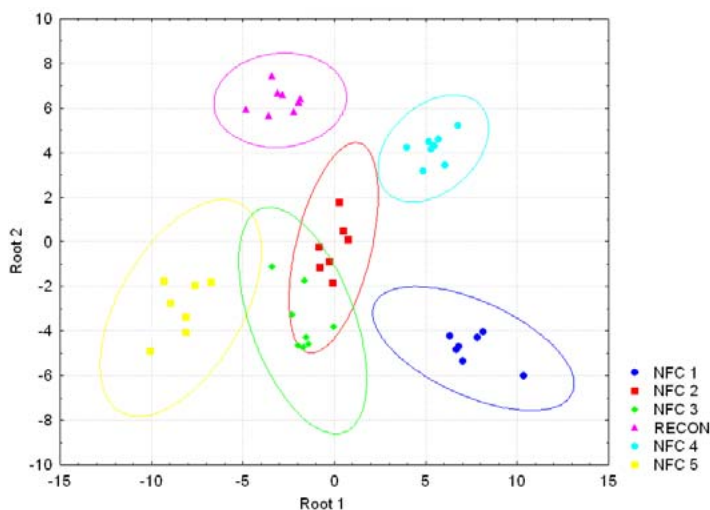

Fig. 1. Discriminant function analysis (DFA) of the electronic nose showing the best results of differentiating 5 not from concentrate and 1 from concentrate orange juices (with replicates).

ent labels). The groupings are different than those produced by the metal oxide electronic nose and have more overlap (Goodner et al., 2001a).

The MS-based electronic nose performed well. Figure 3 shows the results of a DFA of the data with a 3:1 data point to variable ratio (14 variables representing 14 specific m/z). One can see that all the groups are totally separated from each other with the 90% confidence ellipses drawn. The author has previously shown that generally a 3:1 ratio of data points to variables is not generally enough to ensure adequate modeling (Goodner et al., 2001b). It is statistically better to have a higher data point to variable ratio, generally 6:1 or even higher. Given this more stringent requirement, the author re-analyzed only the chemical sensor data to produce an analysis with a data point to variable ratio of 10:1 (4 variables). The results are shown in Figure 4. There is still quite a good separation with four of the products completely differentiated and some overlap of the remaining three (Goodner et al., 2002).
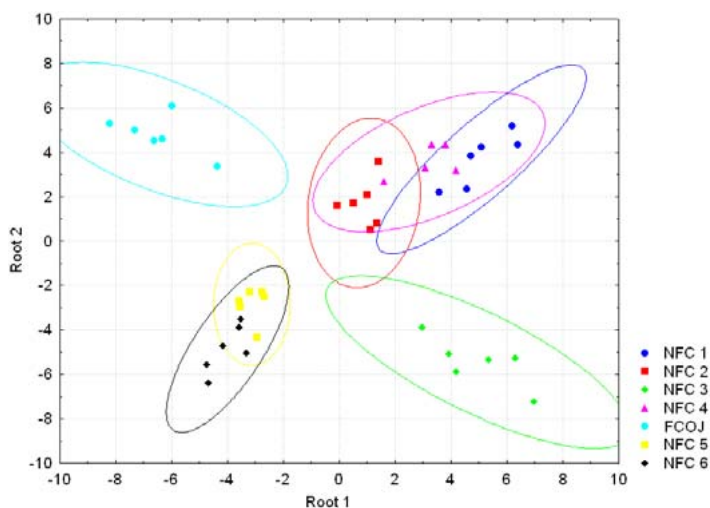

Fig. 2. Discriminant function analysis (DFA) of the HSGC showing the best results of differentiating 6 not from concentrate and 1 from concentrate orange juices (with replicates).
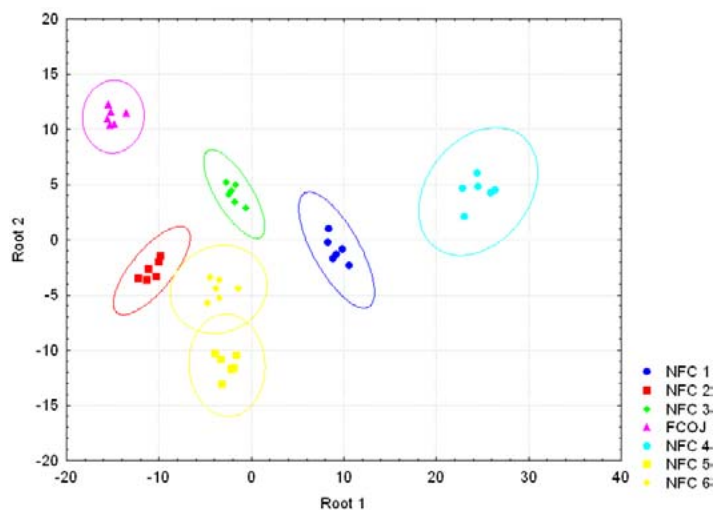
Fig. 3. Discriminant function analysis (DFA) of the MS-based electronic nose with 3:1 data point to variable ratio, the same criteria as Figs. 1 and 2. Results are of differentiating 6 not from concentrate and 1 from concentrate orange juices (with replicates).

Fig. 5. Discriminant function analysis (DFA) of the physical data with 10:1 data point to variable ratio of ten juices. Results of differentiating 7 not from concentrate and 3 from concentrate orange juices (with replicates).

Using routine physical analysis data for multivariate analysis worked very well. Figure 5 shows the results of a DFA for a data set with a 10:1 data point to variable ratio: 10 variables–color (HL, Ha, Hb), °Brix, titratable acidity, ratio, Scott oil, vitamin C, pulp, pH. The data are very well separated with only slight overlap of the 95% ellipses in two cases. In the first overlap, the two samples are both reconstituted orange juice (Recon 1 and 2). In the second overlap, the two samples are juice from the same company, packaged in different containers: paperboard gable-top carton versus PET bottle. The separation seen in Figure 5 is equivalent or better than the other methods.

A DFA of the samples showing separation based on physical analyses on container and processing technique is shown in Figure 6. The not-from-concentrate samples are both clearly separated from each other (different containers) and from the reconstituted (recon) juice products indicating that there is a difference due to container and to processing. The reconstituted (recon) orange juice shows no separation based on

container indicating that there is little difference due to packaging for reconstituted orange juice.

FTIR analysis for the separation of juices by multivariate analysis was shown to work adequately, but does not provide the same levels of separation as the previously discussed data sets. Figure 7 show the results of a DFA for a data set with a 10:1 data point to variable ratio. The variables used for the DFA were the 10 most weighted principal components, which in this study consisted of linear combinations of multiple sensors (wavenumbers). Such transformation of the data by PCA precludes any possibility to identify specific wavenumbers linked to specific classes of compounds responsible for the differentiation of the groups of juices. Juices used to obtain the data presented in Figure 7 are the same as those in Figure 5, but the separation shown in Figure 7 is not as pronounced as that obtained by using the more routine data analyses (as illustrated in Fig. 5). In addition to the poorer separations, PCA analysis of the FTIR data (Fig. 7) presents numerous ad-
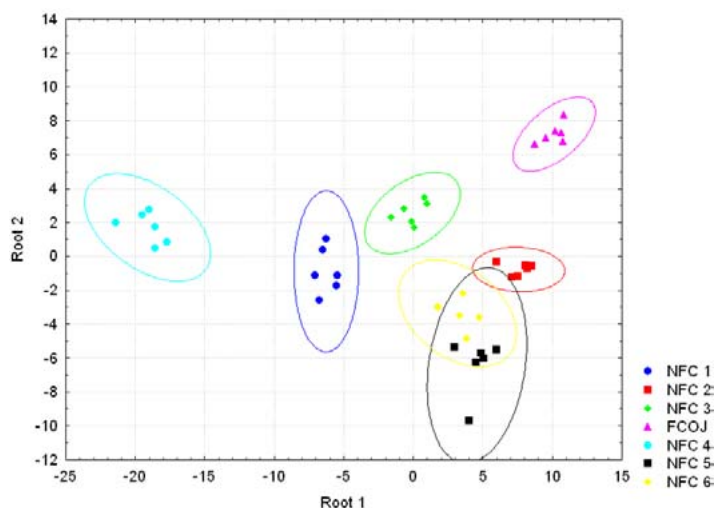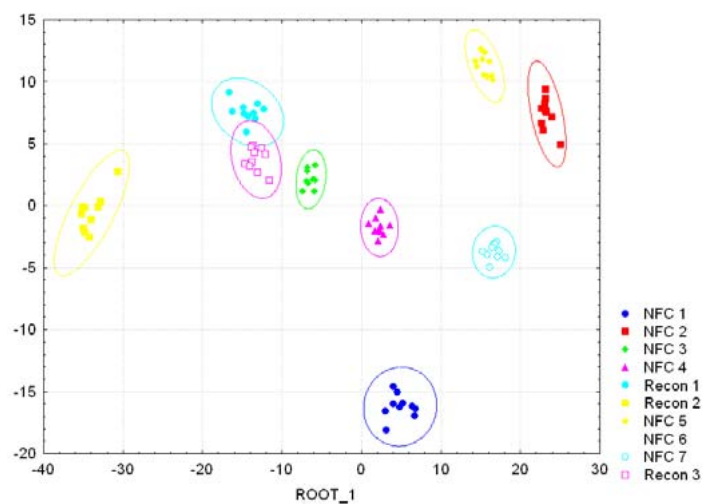

Fig. 4. Discriminant function analysis (DFA) of the MS-based electronic nose with 10:1 data point to variable ratio. Results of differentiating 6 not from concentrate and 1 from concentrate orange juices (with replicates).
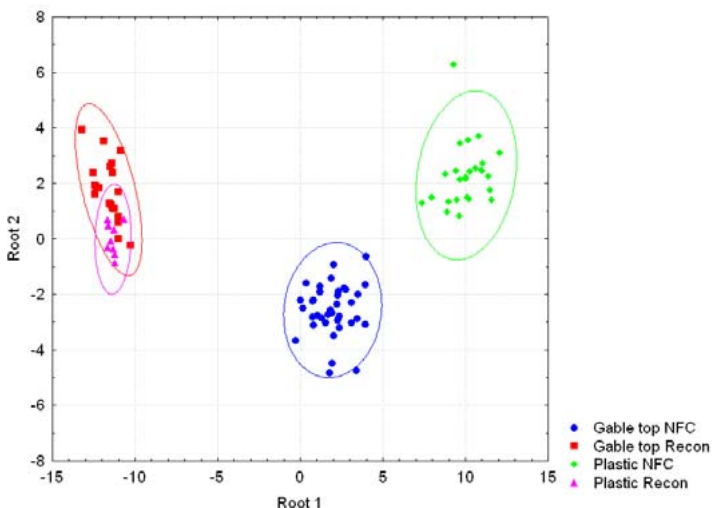

Fig. 6. Discriminant function analysis (DFA) of the physical data with 10:1 data point to variable ratio differentiating container type and processing method.
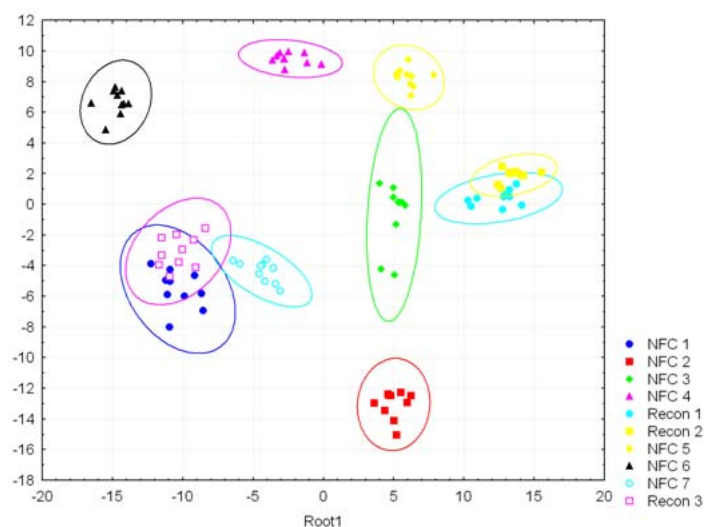
Fig. 7. Discriminant function analysis (DFA) of the FTIR data with 10:1 data point to variable ratio of ten juices. Results of differentiating 7 not from concentrate and 3 from concentrate orange juices (with replicates).

ditional problems associated with software and analysis times for the inordinately large datasets (in this study, 840,000 data points) involved in these studies.
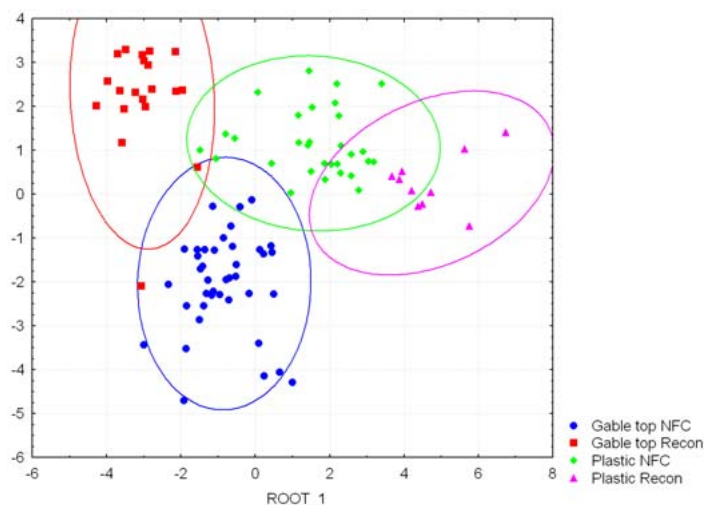

Fig. 8. Discriminant function analysis (DFA) of the FTIR data with 10:1 data point to variable ratio differentiating container type and processing method.

Figure 8 is a graph of the results from FTIR using the same classifications as those in Figure 6, which were based on physical analyses. However, it is clear that Figure 6 is much better. Figure 8 shows little separation of the samples and would not be useful in a general sense. While the FTIR performs adequately for this type of analysis, it is clear from Figures 7 and 8 that there are better choices (i.e., MS-based electronic nose (Fig. 3) and physical/chemical properties (Figs. 5 and 6)) than the FTIR analysis.

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