JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Preliminary Studies for the Differentiation of Apple Juice Samples by Chemometric Analysis of Solid-Phase Microextraction−Gas Chromatographic Data

LINDA M. REID,*,† COLM P. O'DONNELL,† J. DANIEL KELLY,‡ AND GERARD DOWNEY‡

Department of Biosystems Engineering, University College Dublin, Dublin 2, Ireland, and Teagasc, The National Food Centre, Ashtown, Dublin 15, Ireland

A combination of gas chromatography (GC) and chemometrics was evaluated for its ability to differentiate between apple juice samples on the basis of apple variety and applied heat treatment. The heat treatment involved exposure of 15 mL juice samples for 30 s in a 900 W domestic microwave oven. The chromatographic results were subjected to two chemometric procedures: (1) partial least squares (PLS) regression and (2) linear discriminant analysis (LDA) applied to principal component (PC) scores. The percent correct classification of samples were obtained from PLS and LDA in terms of separation on the basis of apple variety and applied heat treatment. PLS gave the highest level of correct classification of the apple juice samples according to both variety and heat treatment, 92.5% correct classification in each case. When LDA was performed on the PC scores obtained from GC analysis, 87.5% and 80% of samples were correctly classified according to apple variety used and applied heat treatment, respectively.

KEYWORDS: SPME-GC; chemometrics; apple juice; differentiation

INTRODUCTION

Apple juice is the second most popular fruit juice consumed in Europe, with 18% of the market share for fruit juices in 2000 (*1*). Like all commercial fruit juices, apple juice requires some form of processing to ensure safety and extend shelf life. Various technologies, such as pulsed electric field processing (*2*), radio frequency processing (*3*), and continuous-flow thermal treatment (*4*), have been investigated for the inactivation of microbes in apple juice.

Microwave treatment has been demonstrated to be effective at pasteurizing apple juice on the basis of its ability to prevent the growth of *Escherichia coli* in juice samples that were inoculated with this bacterium. In an investigation of power levels and treatment times (*5*), the greatest reduction in the growth of *E. coli* in apple juice samples was obtained after treatment for 0.42 min at 900 W. Microwave heating has advantages over more conventional thermal treatments; principal among these is the reduced time exposure to energy, which results in improved product quality.

Solid-phase microextraction (SPME) provides a convenient and relatively rapid means of isolating volatile and semivolatile compounds prior to gas chromatographic (GC) analysis. It involves the absorption/adsorption of volatiles and semivolatiles in a sample matrix onto a fused silica fiber. Direct desorption of the extracted compounds onto a GC column then occurs in the injection port of the GC system. SPME has many advantages over other extraction and preconcentration techniques used with GC (*6*) as it does not require solvents or purge gas and can extract volatile compounds from a sample matrix in a relatively short space of time, usually <30 min. SPME has found many applications in food analysis. In a recent survey of publications dealing with SPME, 20% were concerned with food or botanical analysis (*7*). The various applications of SPME in food analysis have been extensively reviewed (*8*), and its ability to detect characteristic aromas, off-flavors, pesticides, and antibiotics in various foods has been demonstrated.

The application of chemometric techniques to SPME-GC data has been investigated as a means of differentiating food samples on the basis of several criteria. Different strawberry varieties were analyzed by SPME-GC and the chromatographic data were subjected to statistical analysis (*9*). This enabled classification and discrimination of the varieties on the basis of aroma differences. The adulteration of strawberry purée with apple purée has been investigated by use of SPME-GC with principal component analysis (PCA) and partial least squares (PLS) analysis (*10*). Chemometrics in conjunction with SPME-GC have also been applied to differentiation studies on whisky (*11*), coffee (*12*), vegetable oils (*13*), and honey (*14*).

Despite the amount of research carried out to date to differentiate varieties of the same food product, there is relatively little concerning the use of chemometrics on SPME-GC data

* To whom correspondence should be addressed: telephone 00 (353) 1 716 7250; fax 00 (353) 1 475 2119; e-mail Linda.Reid@ucd.ie.
† University College Dublin.
‡ Teagasc, The National Food Centre.

10.1021/jf049143l CCC: $27.50
Published on Web 10/15/2004

to differentiate fruit juice samples on the basis of heat processing and variety of apple used. Two notable exceptions (*15*, *16*) used chemometrics to show that heat-treatment temperature affected the aroma profiles in apple juice samples but did not apply the technology to the differentiation of samples. The differentiation of heat-treated and non-heat-treated juice samples holds obvious applications in the area of food quality assurance and safety.

The objective of this feasibility study was to assess the capability of a combination of gas chromatography (GC) and chemometrics for its potential to differentiate between apple juice samples on the basis of apple variety and applied heat treatment.

MATERIALS AND METHODS

Sample Preparation. Apple juice samples were produced by use of apples collected from small-scale producers throughout Ireland during the 2002 and 2003 harvests. Ten samples each of cv. Jonagold and cv. Bramley were used in the study. Apple juice samples from two different varieties were chosen to determine if changes in the quantity and nature of volatile compounds were greater or less than the inherent differences in such compounds between apple varieties. The samples were juiced on a l'Equip centrifugal juicer, model 110.5 (l'Equip Inc.). Both the flesh and skin of the apples were juiced and the samples were frozen at −20 °C within 4 h of juicing; samples were stored for up to 10 months prior to analysis. Prior to analysis, each apple juice sample was defrosted overnight in a refrigerator at a temperature of 5 °C. On the basis of previous research (*5*), a heating time of 30 s and a power level of 900 W were chosen for the microwave treatment of the apple juice samples; a sample size of 15 mL was used for each heat treatment.

HS-SPME Analysis. A manual SPME holder (Supelco, Bellafonte, PA) was used in all experiments. A 100 μm poly(dimethylsiloxane) (PDMS) fiber (Supelco), 1 cm in length, was used for volatile sequestration. Prior to extraction, the fiber was conditioned for 30 min in the injection port of the GC at 250 °C. Aliquots (5 mL) of the juice samples were transferred to 10 mL headspace (HS) vials. To promote the release of aroma volatiles into the sample headspace, 25% (w/v) NaCl was also added to the sample vials. The vials were crimp-closed with a Teflon-lined silica cap (Supelco) and equilibrated at 60 °C for 15 min with constant stirring. The SPME fiber was exposed to the sample headspace at a constant depth for 20 min. The equilibration conditions used for temperature and agitation were maintained during extraction of the aroma volatiles.

GC Analysis. A Varian 3800 gas chromatography system (Varian Chromatography Systems, Walnut Creek, CA) equipped with a flame ionization detector (FID) and coupled to a Star chromatography software system (v 5.0; Varian Chromatography Systems) was used to perform the analysis. A fused silica capillary column (30 m × 0.25 mm i.d.; Alltech Associates Inc., Deerfield, IL) coated with a 0.25 μm layer of 5% phenyl and 95% poly(dimethylsiloxane) (AT-5) was used. Helium was used as the carrier gas. Thermal desorption of the compounds took place in the GC injection port (equipped with a 0.75 mm i.d. splitless glass liner) at 250 °C for 5 min in splitless mode. The split valve was then opened (1:50) and the fiber remained in the injection port for the entire GC run to ensure complete desorption of the aroma compounds. The detector was operated at 250 °C. The oven temperature was programmed to range from 50 °C (maintained for 3 min) to 250 °C at a rate of 5 °C/ min. The final temperature was maintained for 5 min.

Identification of Headspace Volatiles. GC−mass spectrometry (MS) was carried out to aid in the identification of the headspace volatiles responsible for the greatest amount of variation between samples. The system used was a Varian 3800 GC equipped with a Varian Saturn 2000 ion-trap mass spectrometer (Varian Chromatography Systems). The injection volume was 1 μL and all other conditions were identical to those used for the GC-FID analysis. The mass range studied was m/z 40−650. Compounds were identified by matching their mass spectra with the data stored in the National Institute of Standards and Technology (NIST) library of standard compounds.

Statistical Analysis. To avoid the influence of solvent peaks and evaporation at the start of each chromatographic run, compounds having retention times of <3 min were omitted from the percent peak area calculations. Several pentasiloxane peaks, identified by GC-MS, occurred in the chromatographic runs and were derived from decomposition of the SPME fiber. This has been previously reported as a problem with SPME-GC analysis and prevents the use of the entire GC run for statistical analysis (*17*). Relative peak areas (percent total) were calculated for all resolved GC within the chosen time frame with the exception of the identified pentasiloxane peaks. Analysis of the peak area values for individual peaks in chromatograms pinpointed 18 compounds as significant in discriminations based on either apple variety or heat treatment. Significance in this context was assessed on the basis of increases or decreases in relative peak area with variation in variety and heat treatment. This choice of peaks for chemometric analysis was further supported by subtracting the mean value for relative peak area of the heat-treated samples from the corresponding values for the non-heat-treated samples. All individual peaks chosen for chemometric analysis showed great variation in relative peak area between heat-treated and non-heat-treated.

PCA and PLS were carried out on the chosen compounds by use of The Unscrambler v 7.6 (Camo ASA, Norway) with full cross-validation, via the leave-one-out procedure. The PLS analysis was employed against a nonmetric dummy variable (set to 0 or 1) to test the ability of the method to discriminate between the two different apple varieties and also between heat-treated and non-heat-treated samples; this approach is often referred to as discriminant PLS. The small number of samples used prevented the development of a definitive prediction model for PLS but was still sufficient to enable a preliminary assessment of the potential of the technique to be carried out.

Linear discriminant analysis (LDA) was carried out on PCA scores data by use of Minitab R13.2 (Minitab Inc., State College, PA). PCA sample scores on components 1 and 2, which gave the greatest level of separation in all PCA models used, were input to the LDA analyses. Cross-validation was also carried out on this data set. As with the PLS models, the samples were ascribed dummy variable values of 0 or 1, depending on variety and heat treatment, for the LDA analysis. The LDA results were analyzed in terms of the squared Mahalanobis distance between the two groups being classified and the percentage correct classification of samples.

RESULTS AND DISCUSSION

Figure 1 shows typical chromatograms for volatiles of non-heat-treated and heat-treated Jonagold and Bramley apple juice samples. Not all of the volatile compounds selected for chemometric analysis were capable of being identified by GC-MS, but those that were are included in the caption to **Figure 1**. It is clear that several differences exist between the heat-treated and non-heat-treated juice samples from the comparison of panels A with B and also C with D in **Figure 1**. The differences in compounds present and compound peak areas between the two apple varieties used to produce the juices can also be seen by comparison of panels A and C in **Figure 1**. The 18 compound peak areas selected for chemometric analysis are indicated. It is seen that there are peaks present in the non-heat-treated Jonagold chromatogram (**Figure 1A**) that are not present, or present in only small quantities, in the non-heat-treated Bramley chromatogram (**Figure 1C**) and vice versa. While these peaks may influence differentiation of samples on the basis of variety rather than heat treatment, it was decided to include them in the statistical analysis as their relative peak areas also altered upon heat treatment of the apple juices. An example is peak 6 in **Figure 1A**, not present in Bramley apple juices, which has decreased in area in the heat-treated Jonagold chromatogram in **Figure 1B**. The inclusion of compounds such as these, that were not present in both varieties of apple juice, would be necessary in an industrial application of the technology as commercial apple juices usually contain juices from a wide variety of apples.

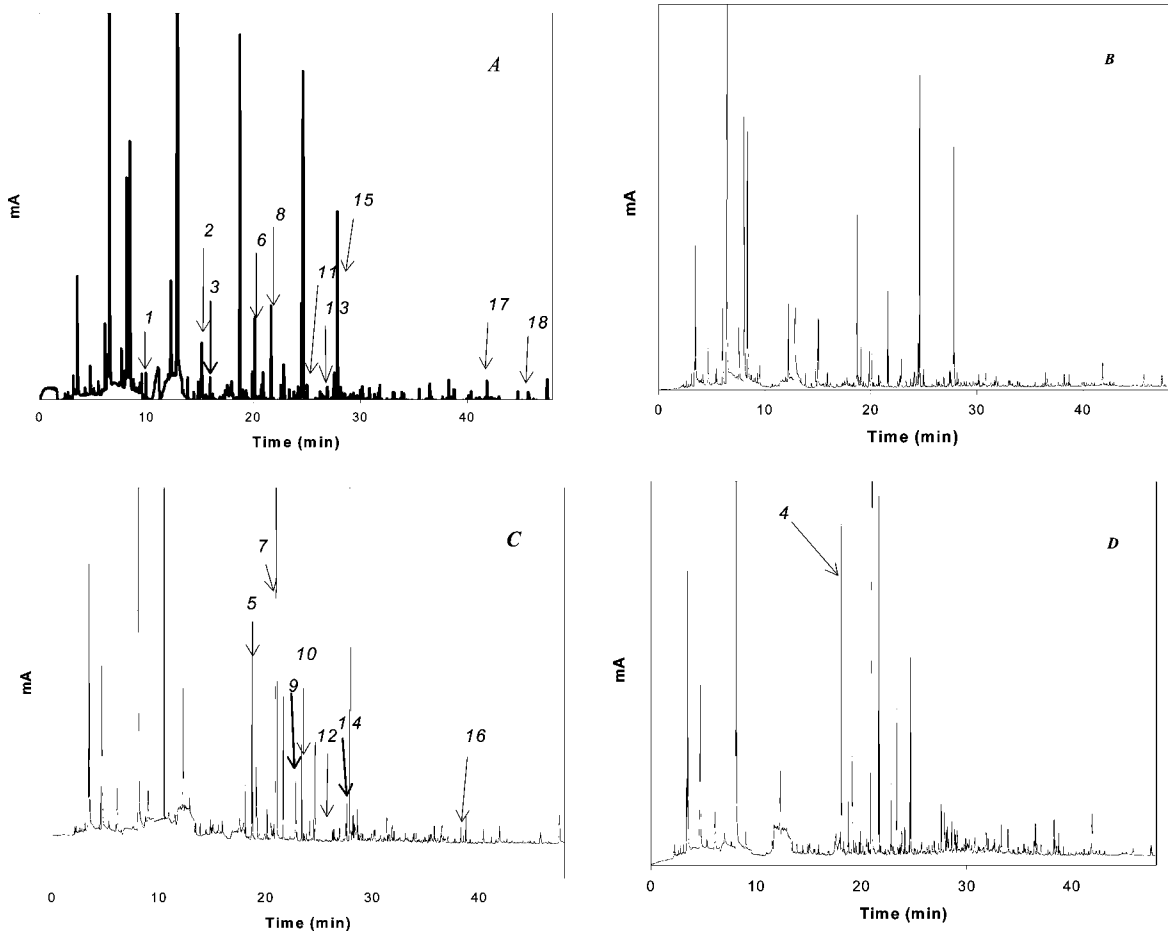

Figure 1. Chromatograms of non-heat-treated Jonagold apple juice (**A**), heat-treated Jonagold apple juice (**B**), non-heat-treated Bramley apple juice (**C**), and heat-treated Bramley apple juice (**D**). The 18 individual peaks that were selected for chemometric analysis are indicated. Those peaks identified by GC/MS were (1) hexyl acetate, (5) 1-decanol, (7) hexanoic acid, (8) hexyl hexanoate; (9) β-ionone, (10) β-caryophyllene, and (15) α-farnesene.

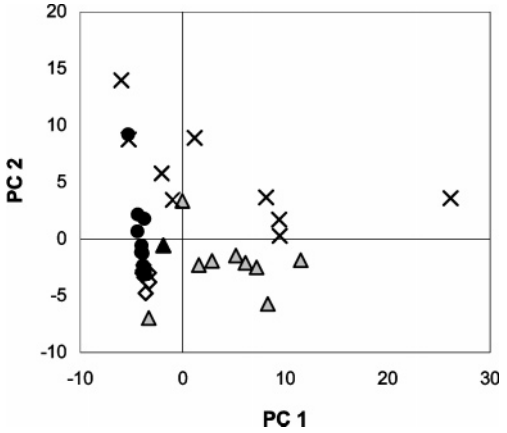

Figure 2. PCA scores plot for the differentiation of apple juice samples on the basis of variety of apple used and applied heat treatment: (◇) Jonagold non-heat-treated; (●) Jonagold heated; (gray △) Bramley non-heat-treated; (×) Bramley heat-treated.

The PCA score plot (PC1 versus PC2) is shown in **Figure 2**. The scores plot reveals that separation along PC1, which accounted for 52% of the variation in the sample set, is chiefly on the basis of apple variety. The Jonagold samples are clustered very tightly about PC1, in marked contrast to the Bramley apples. The spread of these latter samples significantly accounts for PC1. Separation of juice samples along PC2 (which accounted for 24% of variation in the sample set) was on the basis of heat treatment. Most heat-treated samples were situated above the zero value of the PC2 axis, while the majority of non-heat-treated samples were below it. With the exception of a single Jonagold juice, variation in the heat-treated Bramley samples was greater than in the heat-treated Jonagold juices, as indicated by their greater dispersion on PC2. This PC analysis reveals significant structure in the GC data set and suggests that discrimination on the basis of variety and heat treatment may indeed be possible.

The first approach investigated for the discrimination of samples on the basis of variety or heat treatment was discriminant PLS. The PLS regression plots for the GC data are shown in **Figure 3** for variety (panel A) and treatment (panel B). **Figure 3A** reveals that, upon applying a cutoff value of 0.5, one Jonagold sample out of 20 (10 non-heat-treated and 10 heat-treated) and two Bramley samples out of 20 (10 non-heat-treated and 10 heat-treated) were misclassified. This equates to an overall correct classification rate of 92.5%. With regard to discrimination on the basis of heat treatment, **Figure 3B** reveals that one non-heat-treated and two heat-treated samples were misclassified, by use of the 0.5 cutoff value. In this case, the long-term robustness of the model may be less than in the case of variety, since both heat-treatment classes show significant clustering up to the cutoff value. However, this type of behavior has been previously reported for discriminant PLS applications and may be a feature of the procedure (*18*).

The PLS regression coefficients for variety and heat-treatment differentiation by use of GC data are shown in **Figure 4**, panels A and B, respectively. These charts allow the individual compounds with the most influence on the differentiation of

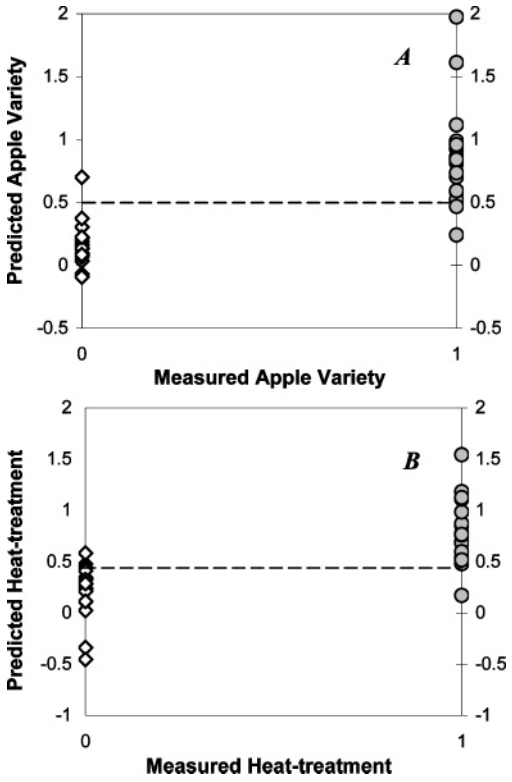

Figure 3. PLS regression plot showing separation of apple juice samples on the basis of apple variety used (**A**) and applied heat treatment (**B**). (**A**) (◇) Jonagold, (gray ○) Bramley; (**B**) (◇) non-heat-treated samples; (gray ○) heat-treated samples.

the samples to be identified. They also allow the direction of influence for a compound to be seen, with positive values for the compounds influencing the component being modeled as 1 and negative values for the component being modeled as zero. **Figure 4A** clearly shows that the main compounds responsible for differentiation on the basis of variety are those that elute at 20.956, 21.628, and 27.84 min. The compound with the retention time of 21.628 min was identified by GC-MS as hexyl hexanoate and that with a retention time of 27.84 min as α-farnesene; the compound eluting at 20.956 min could not be identified by GC-MS. The positive values for these compounds indicate that they are present in greater concentrations in the apple juices made from Bramley apples, as these juices were given the dummy variable of 1 for the PLS analysis. It has been previously reported that α-farnesene is related to sour and bitter taste in apple samples (*19*). This explains its presence in greater amounts in Bramley apples, which are perceived as more sour-tasting than Jonagold. The compound eluting at 9.545 min exhibited the largest negative regression coefficient; this compound was identified by GC-MS as hexyl acetate, which has been previously shown to increase with increased perceived sweetness of apple fruits (*20*). Overall, the compounds with negative values had much smaller regression coefficients, indicating that there was less specific influence by compounds present in the Jonagold samples on the discrimination. This smaller magnitude may also reflect the limited variation in the Jonagold samples, as indicated on the PC scores plot (**Figure 2**).

Figure 4B indicates that the most significant compounds involved in the differentiation of samples on the basis of heat treatment were those with retention times of 18.72 and 27.84 min; these were identified by GC-MS as 1-decanol and α-farnesene, respectively, substances that are recognized as being flavor compounds in apples and apple products (*21*). Both

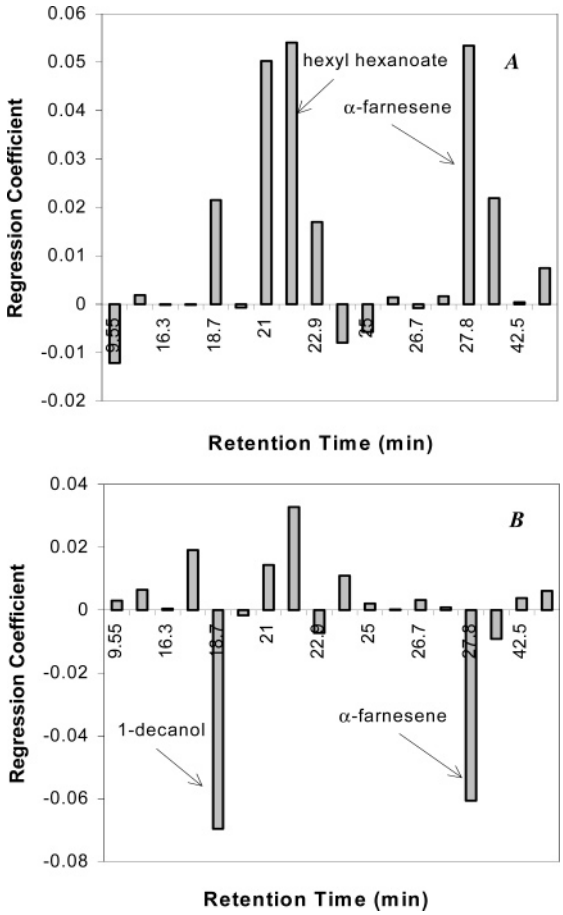

Figure 4. SPME-GC regression coefficients for PLS data showing the influence of individual *X* variables (compound retention times) on the separation of apple juice samples. Results shown are for separation due to apple variety used (**A**) and applied heat treatment (**B**).

Table 1. Percentage Correct Classification Results for LDA and PLS Analysis of the Entire Apple Juice Sample Set

	% correct classification	
discrimination parameter	PLS	LDA
apple variety	92.5	87.5
heat treatment	92.5	80

of these compounds had negative regression coefficient values, indicating that they were present in higher concentrations in the non-heat-treated apple juice samples. By inference, they must be lost to a considerable extent in the heating process. The loss of these compounds due to microwave treatment is also evident from examination of the chromatograms in Figure 1, where 1-decanol is indicated as peak 5 and α-farnesene is indicated as peak 14. The presence of α-farnesene as an important compound in the separation of apple juice samples on the basis of both apple variety and applied heat treatment are indicative of its role as a major flavor compound in apples and apple products.

The PLS results, shown in **Table 1**, show a greater level of correct classification for the apple juice samples, on the basis of both variety and heat treatment, than the LDA results. Correct classification levels of 92.5% the basis of both apple variety and heat treatment were achieved by PLS. LDA, on the other hand, showed corresponding correct classification levels of 87.5% and 80% for separation on the basis of variety of apple and heat treatment, respectively.

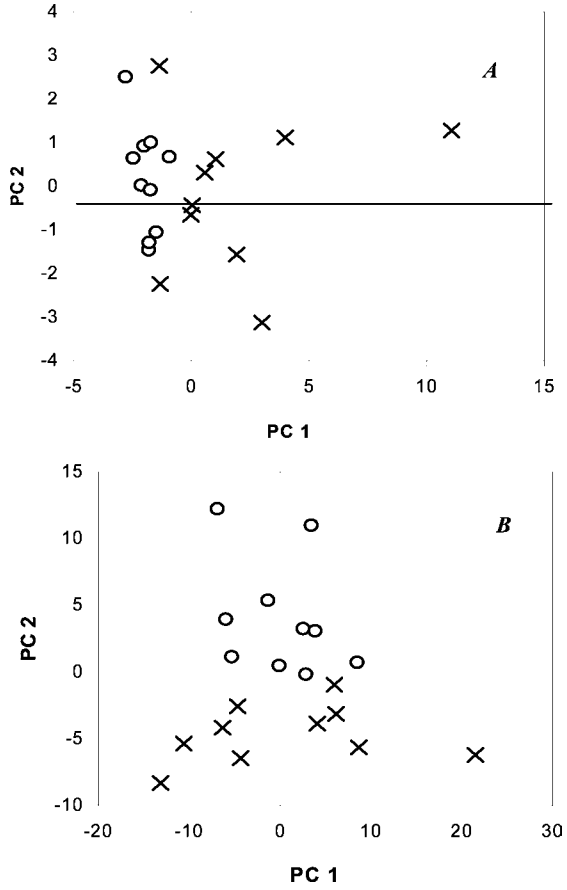

Figure 5. PCA scores plot of individual apple juice varieties for differentiation on the basis of applied heat treatment: (**A**) Jonagold samples; (**B**) Bramley samples; (○) non-heat-treated samples; (×) heat-treated samples.

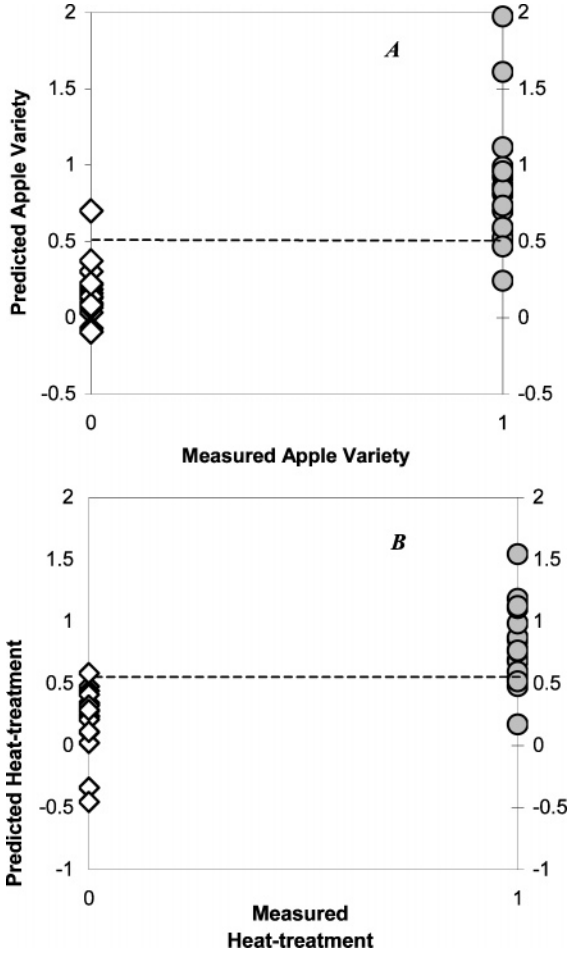

Figure 6. PLS regression plot of individual apple juice varieties showing separation of apple juice samples on the basis of applied heat treatment: (**A**) Jonagold samples; (**B**) Bramley samples.

Chemometric analysis of data from one apple juice variety at a time was carried out to investigate the influence of apple variety on the differentiation between non-heat-treated and heat-treated juice samples. To improve the separation, the individual compound peaks that did not appear in the variety of apple juice being examined were removed from the data set. The PCA scores plot for Jonagold and Bramley apple juice samples are shown in **Figure 5**, panels A and B, respectively. It is clear that there is very good separation of juice samples on the basis of heat treatment in both apple juice varieties. However, while Jonagold juice samples are separated according to heat treatment along the PC1 axis, the Bramley juice samples are separated along the PC2 axis, indicating that there was another variable present in the juice samples that exerted more influence on their separation than heat treatment did.

The level of separation is further illustrated in the PLS regression plots shown in **Figure 6** for Jonagold (panel A) and Bramley (panel B) samples, respectively. In the Jonagold sample set there are three heat-treated juice samples below the cutoff value of 0.5, indicating that these three samples were misclassified by the PLS model. The Bramley sample set, in **Figure 6B**, shows only one misclassified heat-treated sample according to the 0.5 cutoff value. Converting these misclassified samples into values of percent correct classification shows that 85% of Jonagold juice samples and 95% of Bramley juice samples were correctly classified.

The comparison between PLS and LDA for classification of the juice samples is shown in **Table 2**. LDA of the PC scores gave correct classification levels of 90% for both Jonagold juice samples and Bramley juice samples. When these results for correct classification on the basis of heat treatment are compared to those obtained when the entire sample set was studied, it is seen that the classification power of LDA increased when apple juice samples from only one variety were considered, while the classification power of PLS remained almost the same, regardless of whether one or more juices from one or more varieties of apple were considered. This indicates that PLS is less sensitive to the presence of apple juices from different varieties of apple and also that PLS analysis of the SPME-GC data may be more useful in an industrial setting, where it is unlikely that juice from a single variety of apple would be used.

Table 2. Percentage Correct Classification Results for LDA and PLS Analysis of Juice Samples from Individual Apple Varieties

	% correct classification			
	Jonagold		Bramley	
discrimination parameter	PLS	LDA	PLS	LDA
heat treatment	85	90	95	90

Due to the small sample size, it is not possible to obtain a definitive picture of the capabilities of this approach for the detection of heat treatment in apple juices. However, the results for this feasibility study clearly show the potential for the application of GC with PLS and LDA for the detection of heat treatment in apple juices. This parameter was overshadowed by the influence of apple variety used in the production of juice

samples. As the detection of heat treatment holds more importance in terms of food safety issues, it appears that future work should focus on overcoming the influence of apple variety. Analysis of juice samples that contain juice from a number of different apple varieties would also give a clearer indication of the suitability of this approach for use in an industrial setting. This could potentially enable the development of a protocol based on the analytical and chemometric methods used in this research to detect juice samples that did not receive sufficient heat treatment to ensure the desired shelf life of the juice.

ACKNOWLEDGMENT

We acknowledge Jennie Moriarty and John Wenger, Department of Chemistry, University College Cork, Ireland, for their assistance with the GC/MS analysis.

LITERATURE CITED

(1) *Fruit juice market in Europe*; Zenith International: Bath, U.K., 2001.
(2) Zarate-Rodriguez, E.; Ortega-Rivas, E.; Barbosa-Canovas, G. V. Quality changes in apple juice as related to nonthermal processing. *J. Food Qual.* **2000**, *23* (3), 337−349.
(3) Geveke, D. J.; Kozempel, M.; Scullen, O. O.; Brunkhorst, C. Radio frequency energy effects on microorganisms in foods. *Inn. Food Sci., & Em. Tech.* **2002**, *3*, 133−138.
(4) Shimoda, M.; Katoh, T.; Suzuki, J.; Kawaraya, A.; Igura, N.; Hayakawa, I. Changes in the odours of reconstituted apple juice during thermal processing. *Food Res. Int.* **2003**, *36*, 439−445.
(5) Cañumir, J. A.; Celis, J. E.; de Bruijn, J.; Vidal, L. V. Pasteurisation of apple juice using microwaves. *Food Sci. Technol.* **2002**, *35*, 389−392.
(6) Harmon, A. D. Solid-phase microextraction for the analysis of flavors. *Techniques for analyzing food aroma*; Marsili, R., Ed.; Marcel Dekker: New York, 1997.
(7) Pillonel, L.; Bosset, J. O.; Tabacchi, R. Rapid preconcentration and enrichment techniques for the analysis of a food volatile—A review. *Food Sci. Technol.*. **2002**, *35*, 1−14.
(8) Kataoka, H.; Lord, H. L.; Pawliszyn, J. Applications of solid-phase microextraction in food analysis. *J. Chromatogr. A* **2000**, *880*, 35−62.
(9) Urruty, L.; Giraudel, J.-L.; Lek, S.; Roudeillac, P.; Montury, M. Assessment of strawberry aroma through SPME/GC and ANN methods. Classification and discrimination of varieties. *J. Agric. Food Chem.* **2002**, *50*, 3129−3136.
(10) Reid, L. M.; O'Donnell, C. P.; Downey, G. The potential of SPME-GC and chemometrics to detect adulteration of fruit purées. *J. Agric. Food Chem.* **2004**, *52*, 421−427.
(11) Lee, K. Y. M.; Paterson, A.; Birkmyre, L.; Piggott, J. R. Headspace congeners of blended Scotch whiskies of different product categories from SPME analysis. *J. Inst. Brew.* **2001**, *107*, 315−332.
(12) Bicchi, C. P.; Panero, O. M.; Pellegrino, G. M.; Vanni, A. C. Characterization of roasted coffee and coffee beverages by SPME-GC and PCA. *J. Agric. Food Chem.* **1997**, *45*, 4680−4686.
(13) Jelén, H. H.; Obuchowska, M.; Zawirska-Wojtasiak, R.; Wasowicz., E. HS−SPME use for the characterization of volatile compounds in vegetable oils of different sensory quality. *J. Agric. Food Chem.* **2000**, *48*, 2360−2367.
(14) Peréz, R. A.; Sánchez-Brunete, C.; Calvo, R. M.; Tadeo, J. L. Analysis of volatiles from Spanish honeys by SPME and GC-MS. *J. Agric. Food Chem.* **2002**, *50*, 2633−2637.
(15) Kato, T.; Shimoda, M.; Suzuki, J.; Kawaraya, A.; Igura, N.; Hayakawa, I. Changes in the odours of squeezed apple juice during thermal processing. *Food Res. Int.* **2003**, *36*, 777−785.
(16) Su, S. K.; Wiley, R. C. Changes in apple juice flavor compounds during processing. *J. Food Sci.* **1998**, *63*, 688−691.
(17) Tura, D.; Prenzler, P. D.; et al. Varietal and processing effects on the volatile profile of Australian olive oils. *Food Chem.* **2004**, *84*, 341−349.
(18) Downey, G. Discrimination strategies in food authentication—what is the correct question to ask? *MIR Newsletter* **2000**, *11* (6), 8−9.
(19) Karlsen, A. M.; Aaby, K.; Sivertsen, H.; Baardseth, P.; Ellekjaer, M. R. Instrumental and sensory analysis of fresh Norwegian and imported apples. *Food Qual. Pref.* **2002**, *10* (4−5), 305−314.
(20) Young, H.; Gilbert, J. M.; Murray, S. H.; Ball, R. D. Causal effects of aroma compounds on Royal Gala apple flavours. *J. Sci. Food Agric.* **1996**, *71* (3), 329−336.
(21) Ibáñez, E.; López-Sebastián, S.; Ramos, E.; Tabera, J.; Reglero, G. Analysis of volatile fruit components by HS−SPME. *Food Chem.* **1998**, *63* (2), 281−286.

Received for review May 27, 2004. Revised manuscript received August 25, 2004. Accepted September 5, 2004. This research was funded under the Food Institutional Research Measure administered by the Irish Department of Agriculture and Food.

JF049143L