Characterization of Royal Gala Apple Aroma Using Electronic Nose Technology—Potential Maturity Indicator

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Changes in aroma of apple harvested at four different maturities were measured at harvest and after short-term storage using electronic aroma sensors ("electronic nose") and classical headspace/gas chromatography methods. Stored fruits were also evaluated by a trained sensory panel. Compared with headspace/gas chromatography, the electronic nose was found to be more sensitive (~40 times) in terms of sample size. The sampling procedure for the electronic nose was much less complex. Using discriminant function analysis, both methods classified the apples tested into groups according to harvest date. After storage, the groupings were more diffuse. Results from sensory testing showed partial separation along the first linear discriminant but did not classify the apple into distinct groups. Important differences between treatments were found for "overall flavor", "acid flavor" intensity, "crispness", "cider/fermented aroma", "vegetative aroma", and "canned pear aroma".

Keywords: *Electronic nose; apple; maturity; flavor; sensory analysis; GC/MS*

INTRODUCTION

Maturity at harvest can have a dramatic effect on the sensory and storage properties of apples (Jager et al., 1996). Currently several properties, including background color, starch conversion, degrees Brix, and firmness testing, are used in the apple industry to determine optimal harvest dates, and depending on the variety and country of origin, some or all of these properties are used as an index of maturity. Harker et al. (1992) noted that the relationship between background color and fruit maturity of Royal Gala apples in New Zealand is not consistent. Nevertheless, it is one of the better indicators of maturity for New Zealand grown apples (Watkins et al., 1993). Although the flavor volatile profile changes as the apple fruit progresses through maturation, harvest, and subsequent storage (Song et al., 1994), it has not been used as a maturity indicator by industry. Classical methods of flavor volatile analysis, such as gas chromatography (GC), alone or together with mass spectrometry (GC/MS), require expensive instrumentation and are difficult to carry out. Over 200 volatile components have been identified for apples (Dimick and Hoskin, 1983). Young et al. (1996) identified 30 compounds in the flavor volatiles of Royal Gala apples, whereas Mattheis et al. (1998) identified 20 esters from intact fruit of Gala apple. This makes the interpretation of the resulting dataset a difficult task, although only a few of these compounds may have causal effects on the sensory attributes of aroma and flavor. Even if only the major contributors to sensory attributes, for example, 2-methylbutyl acetate, butanol, and hexyl acetate in the case of Royal Gala apple (*Malus domestica* Borkh.) (Young et al., 1996), are considered, a skilled analyst is still required to measure these three components by headspace/GC methodology.

Instruments employing multiple sensors combined with pattern recognition software have become available commercially as an "electronic nose". There have been several reports of electronic sensing of aroma from fruit. Simon et al. (1996) used tin oxide based gas sensors to monitor blueberry aroma. They were able to distinguish low levels of damaged fruit in package containers and levels of fruit ripeness ranging from mature-green to ripe fruit. Ripeness in melons using electronic aroma sensing derived data has been related to various ripeness indices such as external color, slip pressure, and classical volatile measurements (Benady et al., 1992). Electronic nose technology could overcome some of the difficulties associated with classical measurement of flavor volatiles for use as harvest, or other, maturity indices. The aim of this study is to determine the applicability of the electronic nose, in terms of selectivity and sensitivity, for the measurement of aroma in apples with only small differences in sensory properties. Results are compared with headspace/GC data.

MATERIALS AND METHODS

Fruit. Royal Gala apples were harvested from the HortResearch orchard in Hawkes Bay, New Zealand, during the 1998 commercial harvest season and were transported overnight to the Mt. Albert Research Centre. Fruits were from four commercial harvests and are designated early 1 (February 17), early 2 (February 19), mid (February 27), and late (March 12). Average background color index and storage time for early 1 are 2.85 and 6 weeks, for early 2, 4.05 and 6 weeks, for mid, 5.1 and 4 weeks, and for late, 6.2 and 2 weeks. Background color index is estimated using color charts appropriate for Royal Gala (0 = green and 6 = yellow).

On arrival at the Mt. Albert Research Centre the fruit were kept at 0 °C until sampled. Fruits were sampled by electronic nose and headspace/GC at harvest (within 3 days of storage) after storage. Prior to sampling, fruits were removed from cool storage and allowed to warm to ambient temperature (20 °C) overnight. For each treatment five fruits were examined individually; half of the fruit was used for headspace measurement and the other half used for testing on the electronic nose.

GC and MS. An HP-5890 gas chromatograph was used with a DB-Wax fused silica capillary column (30 m × 0.32 mm i.d. × 0.5 μm film; J&W Scientific, Folsom, CA); H_2 carrier gas was controlled using an EPC1000 electronic pressure controller

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(Alltech, Deerfield, IL) (74.4 kPa for 30 min, 6.89 kPa/min to 143.3 kPa, 12 min hold; initial flow rate was 30 cm/s) and flame ionization detector temperature of 210 °C. The column temperature program was 30 °C, hold for 6 min, and 3 °C/min to 190 °C. Samples were desorbed (175 °C) from the Chromosorb 105 traps onto the column using cryofocusing. Peak areas were converted to mass using an average response factor based on ethyl butanoate, butyl acetate, 2-methylbutyl acetate, butanol, ethyl hexanoate, and hexanol, major components found in Royal Gala apples (Young et al., 1996). GC/MS was carried out on a VG 70-SE mass spectrometer (Micromass U.K. Ltd., Manchester, U.K.) using the same column and conditions as for GC but with He as the carrier gas. Peak identification was carried out using retention indices aided with GC/MS data.

Headspace Sampling for GC Analysis. A cylinder of apple cortical tissue (10 mm diameter × 20 mm long) was placed in a 50 mL test tube (Quickfit) fitted with an air inlet and outlet head, and the test tube was placed in a 30 °C water bath. Inlet air was directed to the bottom of the test tube using a short length of Teflon tubing. A stainless steel cartridge packed with Chromosorb 105 (100 mg) (Young, 1981) was fitted to the outlet for headspace volatile collection. Purified air was passed through the apparatus at 25 mL/min for 20 min. Each fruit was sampled in duplicate.

Electronic Nose. Samples were analyzed on a Fox 4000 system (Alpha MOS, France) fitted with P30/1, P10/1, P10/2, P40/1, P40/2, PA3, P70/0, T50/3, PA2, T50/1, T40/1, T70/2, SY/LG, SY/G, SY-cG, SY-gW, SY-W, and SY-gCT sensors. The electrical output from the sensors was measured at 1 s intervals. Samples were introduced using a flow-through method. Humidified air (bubbled through saturated aqueous K_2SO_4 at 30 °C) at 150 mL/min was used as the sweep gas. One disk of apple cortical tissue (5 mm thick × 10 mm diameter) was placed in a glass jar (120 mL) with gas inlet and outlet tubes fitted through the lid. The jar was equilibrated in a 30 °C air oven prior to use. Sampling was carried out at 30 °C with an initial 3 min purge and 10 min aroma generation time. The electronic nose data acquisition program was 30 s sensor pre-equilibrium time and 60 s actual sampling time followed by a 510 s delay for sensor recovery. A 100 μL aliquot of an aqueous solution containing ethyl butanoate, butyl acetate, 2-methylbutyl acetate, butanol, ethyl hexanoate, and hexanol at 0.46, 0.50, 0.50, 0.42, 0.42, and 0.43 μg/mL, respectively, was used to calibrate the sensors before use each day. Calibration was carried out using the manufacturer-supplied software.

Sensory Evaluation. Sensory testing was carried out on stored fruit. Aroma, flavor, and texture were profiled by 16 trained panelists with previous experience of descriptive profiling. Training was carried out over 4 days. In the first training session panelists generated a list of aroma, flavor, and texture descriptors following sampling of the fruit. This exercise was followed by a group discussion in which the generated descriptors were discussed. The following three training sessions focused on defining the attributes and selecting suitable reference standards (Table 1). All sensory assessments took place at HortResearch's Sensory Science Facility at the Mt. Albert Research Centre, Auckland. The booth environment was held at 20 °C and provided with a positive air flow to remove odors from the testing area. Filtered water (Microlene) and plain water crackers were provided as palate cleansers. Panelists were required to assess the fruit and indicate the intensity of each of the descriptors on 150 mm unstructured line scales, where 0 = absent and 150 = extreme. In addition, panelists were instructed to describe any other flavor terms present in the samples. Each panelist was presented with a peeled half fruit of each of the four samples according to a randomized balanced complete block design. Attribute definition sheets with line scales showing the position of reference standards were provided.

Statistical Analysis. Sensory data were analyzed using residual maximum likelihood (REML). To compare statistical differences between means, Tukey's least significant difference (LSD) values at $p \leq 0.05$ were calculated from the standard error of difference. Analysis of variance on the GC data was carried using Genstat. The S-Plus statistical package was used for discriminant function analysis (DFA). Principal component analysis (PCA) for the electronic nose data was carried out using the built-in statistical functions of the Fox 4000.

Table 1. Attribute Definitions Used in Sensory Profiling of Royal Gala Apples

descriptor	definition
cider/fermented aroma	aroma of fermented apples
canned pear aroma	aroma of canned pears
vegetative aroma	aroma of freshly cut grass
stalky aroma	grassy, woody, apple pips/core flavor
metallic flavor	flavor of metal
honey flavor	flavor of creamed honey
cooked apple flavor	flavor of stewed apples
lemon flavor	flavor of a freshly squeezed lemon
acid	taste sensation caused by acids
sweet	taste sensation caused by sugars
overall flavor	initial impression of the strength of the overall flavor present
astringency	sensation caused by drying of the tongue and puckering of the cheeks
crispness	amount and pitch of sound generated on the first bite of sample with the front teeth
juiciness	amount of juice released from the sample when chewed with the back teeth
pulpiness	amount of wet, weblike material that develops during chewing

RESULTS AND DISCUSSION

The flavor volatile profile of Royal Gala apples was determined by electronic nose and by dynamic headspace followed by GC analysis. Early 1 and early 2 fruits were chosen to test the selectivity of the electronic nose in distinguishing small changes, which are expected at the beginning of commercial harvest. A key objective of this study was to test if electronic nose technology is capable of differentiating fruits that are different from a sensory viewpoint. To remove day effects in the sensory data, a variable storage time was chosen.

PCA results for the electronic nose data recorded at 120 s are shown in Figure 1. Data taken at the 100 and 150 s time intervals were also examined with results similar to those found at 120 s and will not be considered in this paper. Using PCA (Figure 1), no clear groupings are apparent between treatments, but there is a clear trend along PC 1, with the late harvest fruit removed from the other harvests. There is very little distinction between early 1, picked before commercial harvest criteria were met, and early 2 harvest, which were picked 2 days later. When DFA, using four groups (early 1, early 2, mid, and late) was applied to the same data set, early 1 and early 2 fruits were clustered together but the mid and late fruits were clearly distinguishable from each other and from the early 1/early 2 fruits (Figure 2a). DFA function 1 and function 2 accounted for 70 and 26% of the variance, respectively. The cross-validation classification for early 1 had 5 (n = 10) classified into early 2, whereas for early 2 fruit 9 (n = 10) were correctly classified. Both mid and late fruits were all classified into their respective groups.

DFA results for the after-storage electronic nose data are shown in Figure 2b. As found for the at-harvest fruit, four groups corresponding to the harvest maturity are found. However, this time the points within groups are not as tightly clustered. DFA function 1 and function 2 accounted for 65 and 27% of the variance, respectively. Cross-validation had 3 (n = 7) early 1 classified into

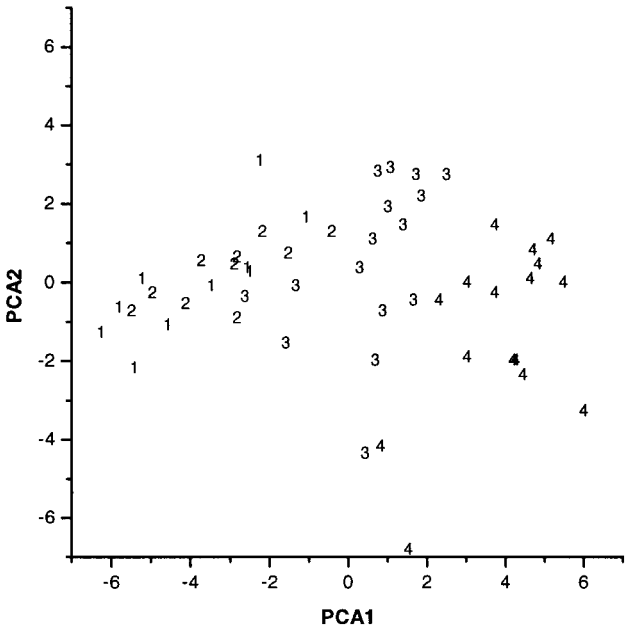

Figure 1. PCA results of electronic nose data taken at 120 s from Royal Gala apple aroma at harvest. Each point represents one measurement from an individual apple. Replicates are not identified. 1, early 1; 2, early 2; 3, mid; 4, late.

early 2, wheres 4 early 2 fruits were classified into early 1. For the mid fruit ($n = 10$), one was classified as early 2 and one as late. Late fruit had one ($n = 10$) classified as mid.

Abundant volatile components collected (headspace/GC data) from fruit at harvest and after storage are shown in Figure 3, parts a and b, respectively (Young et al., 1996). Other minor components found, both at harvest and after storage, were (*Z*)-3-hexenyl acetate (1.3 ng/g, LSD = 1.7; 0.5 ng/g, LSD = 1.9), (*Z*)-3-hexen-1-ol (2.9 ng/g, LSD = 5; 2.11 ng/g, LSD = 4.2), acetic acid (4.4 ng/g, LSD = 5.2; 3.15 ng/g, LSD = 4.1), and 3-hexenal (not detected at harvest; 4.7 ng/g, LSD = 7.9).

Butyl, 2-methylbutyl, and hexyl acetate levels at harvest increased with increasing maturity, although these increases were only significant in the late harvest. Aldehyde levels show little change over the four harvest dates. Butanol levels were higher for the mid and late harvest fruits compared with the two early harvest fruits. 2-Methylbutanol showed an increase at the late harvest, whereas hexanol levels were relatively constant over the four harvests.

DFA of the at-harvest GC dataset is shown in Figure 4a. Fruits were clustered in a fashion similar to that found using electronic nose data. However, with the GC data there was greater scatter within a group. DFA function 1 accounted for 69% of the variance, whereas function 2 accounted for 27%. Cross-validation check had two ($n = 10$) early 1 fruits classified as early 2 and four early 2 fruits classified as early 1. Mid and late fruits were all correctly identified. A possible contribution to the more random nature of the data for the GC dataset could be the more complex sampling procedure required to collect the volatiles and the difficulty of assessing peak area accurately for very small peaks, such as those for the hexenyl acetates.

Physiological changes that occur during storage are apparent in the after-storage GC measurements. Butyl and 2-methylbutyl acetate levels had not changed significantly for the early 1 and early 2 fruits but had

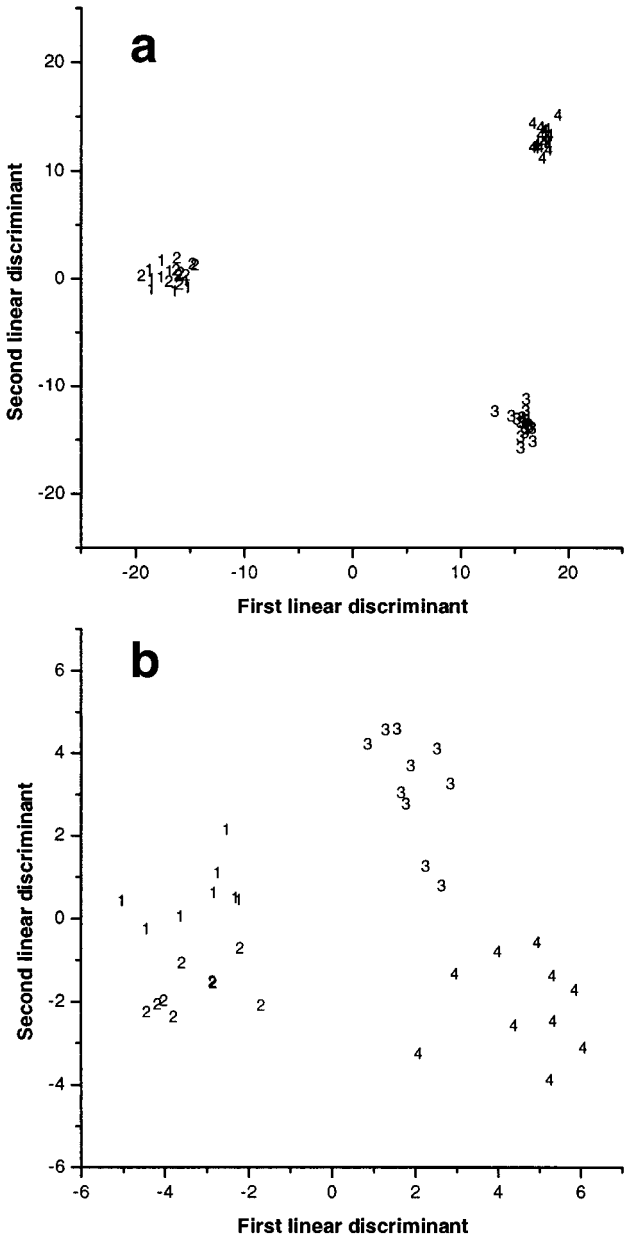

Figure 2. DFA results of electronic nose data taken at 120 s from Royal Gala apple aroma at harvest (a) and after storage (b). Each point represents one measurement from an individual apple. Replicates are not identified. 1, early 1; 2, early 2; 3, mid; 4, late.

decreased for mid and late harvest fruits. Butanol increased in late harvest fruit; otherwise, the alcohol levels were similar to those found at harvest. Aldehyde levels, hexanal and (*E*)-2-hexenal, were similar to those at harvest. DFA of the dataset of stored fruit showed even more scattering within groups. Early 1, early 2, and mid fruits were not separated, although there was a trend along the second discriminant (Figure 4b). Cross-validation tests showed good classification of early 1 fruit [with only one ($n = 15$) placed into early 2] and late fruit [with one ($n = 15$) placed into mid]. Results for early 2 and mid were less rigorous. Early 2 fruit ($n = 15$) had two in early 1 and four in the mid group, whereas the mid fruit had three in early 1 and two in early 2 groups.

The electronic nose demonstrated several advantages over headspace/GC analysis if chemical identification of the volatile components is not required. In terms of

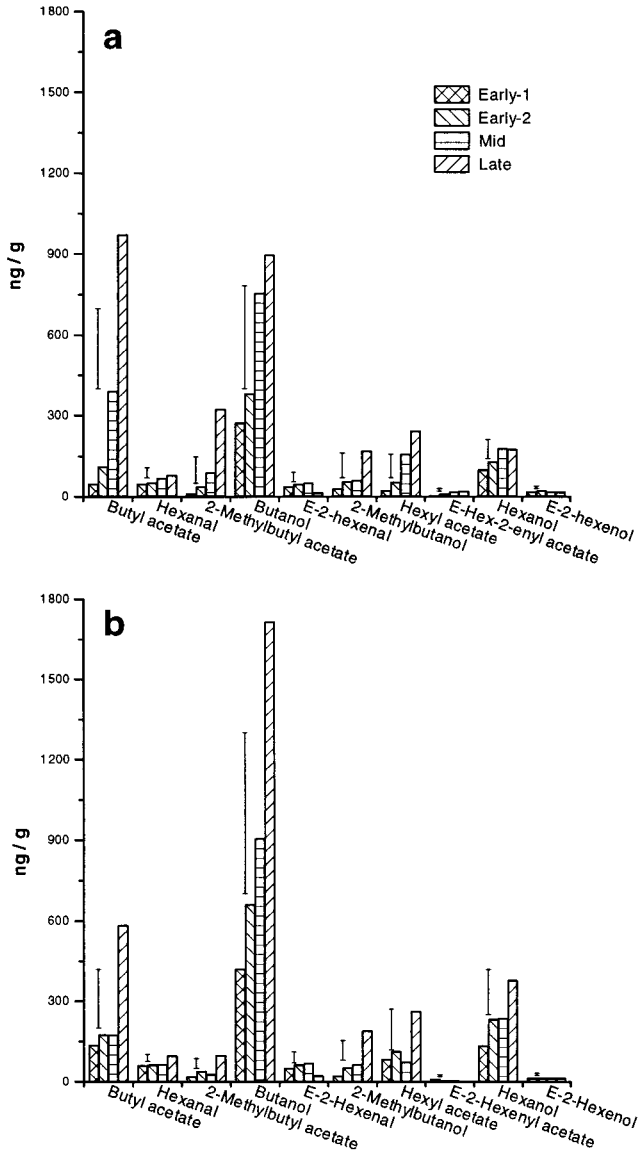

Figure 3. Major aroma components released by Royal Gala apple at harvest (a) and after storage (b).

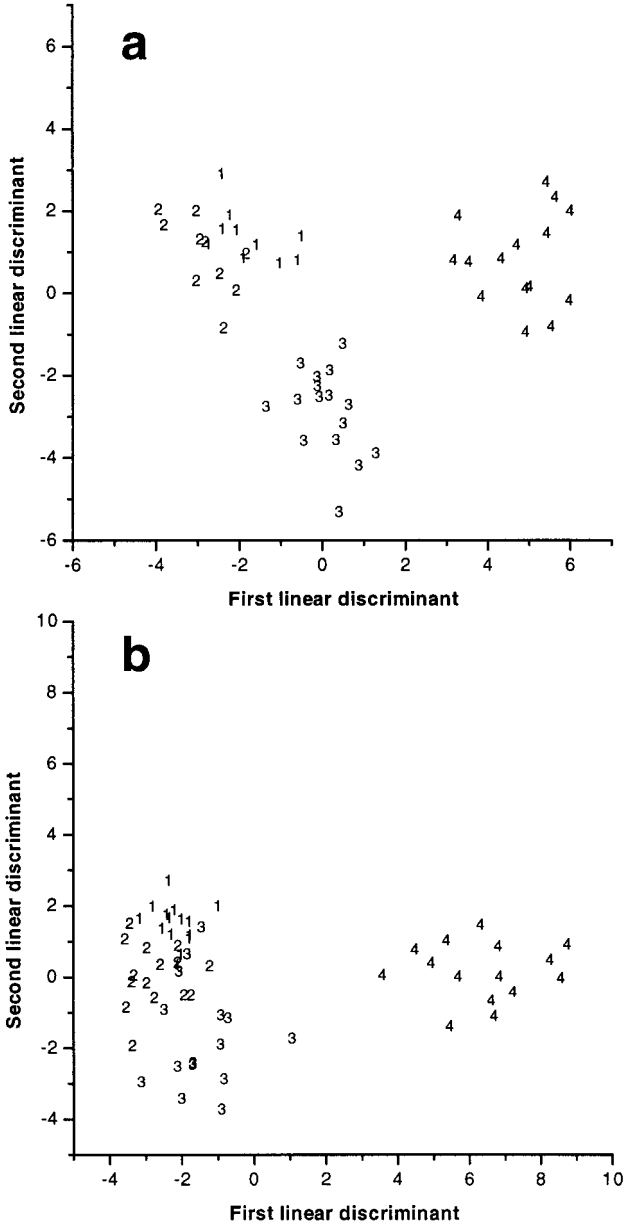

Figure 4. DFA results of headspace/GC data from Royal Gala apple aroma at harvest (a) and after storage (b). Each point represents one measurement from an individual apple. Replicates are not identified. 1, early 1; 2, early 2; 3, mid; 4, late.

sample size, the sensitivity of the electronic nose is ~40 times greater than that of the headspace/GC method, although the latter could be made more sensitive by more extensive crushing of the apple tissue (data not shown). The electronic nose has a much simpler sample preparation, in both time and complexity. Sample throughput for the electronic nose was 15−20 min, whereas GC analysis required 50−60 min. Most of the time required for the electronic nose was for sensor recovery. A different sensor technology may be able to reduce this recovery period considerably. In this experiment the GC conditions were chosen to maximize separation of the components. Using more advanced pattern recognition procedures and "fast" GC, significantly shorter run times may be possible as complete separation of the components may not be necessary.

Sensory testing did not find any extreme differences among the four harvest dates for the descriptors listed in Table 1. The largest difference was found in late harvest fruit, which had significantly less "vegetative aroma", more "canned pear aroma", lower acidity, and lower crispness. Early 2 fruit scored the greatest "overall flavor intensity" and was the sweetest of all harvests (Table 2). The highest acid fruits were the early 1 fruits. DFA of the sensory data was only partially successful in classifying the various harvests (Figure 5), although a trend was apparent along function 1. No classification of the early 1 and early 2 fruits was shown. Compared with electronic nose and GC data, sensory results show even more scattering than the GC data. It should be noted that the sensory data included texture and contributions from nonvolatile components such as the sugars and acids, whereas the electronic nose and the headspace procedures are sensitive only to the aroma components.

These results have demonstrated that electronic aroma sensing technology has excellent sensitivity and selectivity for differentiating Royal Gala apple on the basis of harvest date. With the electronic nose, monitoring of volatile mixtures is greatly simplified. The changing aroma profile as the apple matures (Song et al.,

Table 2. Mean Scores, Estimated Using REML, for Sensory Evaluation of Royal Gala Apples by Analytical Panelists[a]

	harvest					
attribute	early 1	early 2	mid	late	significance	LSD[b]
cider/fermented aroma	47.6b	53.8ab	63.4ab	69.9a	*	20.36
canned pear aroma	33.1b	35.1b	42.7ab	61a	*	22.38
vegetative aroma	70.9a	68.8a	62.5ab	43.7b	*	21.08
stalky aroma	58.7	53.7	51.4	48.4		17.77
overall flavor	77.7ab	91.6a	81.1ab	71.2b	*	20.38
honey flavor	46.5	42.4	46.7	51.6		19.10
cooked apple flavor	54.2	50.4	43.5	54.3		19.29
lemon flavor	43.9	39.8	37.6	30		19.31
acid taste intensity	71.1a	69.9a	61.2ab	44.2b	*	21.21
sweet taste intensity	63.9	71.4	70.9	63		15.66
crispness	86.7a	88.2a	71.99a	67.1b	*	15.79
juiciness	88	84.7	87	75.3		16.25
pulpiness	77.2	65.4	71.9	72.4		16.57
astringency	35.8	34.6	33.7	26.9		21.48

[a] Scores were registered on a 150 mm line scale, and there were 16 assessments per treatment. [b] Tukey's LSD, $p < 0.05$. Means in the same row, followed by the same letter, are not significantly different.

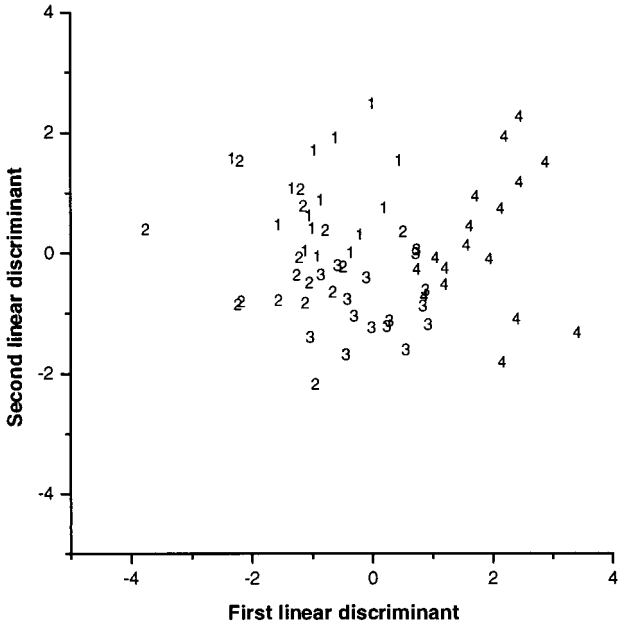

Figure 5. DFA results of sensory data for Royal Gala apple after storage. Replicates are not identified. 1, early 1; 2, early 2; 3, mid; 4, late.

1994) can potentially be a useful addition to the range of objective measurements currently employed to determine harvest dates of apple for optimal storage life and eating quality. In this paper DFA was used to classify the four maturity classes but the application of an artificial neural network could potentially provide answers in real time, especially as sensors with faster response time become available. Calibration was carried out in this study to minimize the effects of drift and changes in sensor sensitivity as measurements were carried out over several weeks. However, electronic aroma sensing is a relatively new technology for which there are no established methods for checking performance and long-term stability (Mielle, 1996).

In this study we have measured sensory quality after only a short storage period and have not considered the relationship of aroma volatiles at harvest as a predictor of quality after longer storage or as a measure of sensory quality after longer term storage. These results show that the measurement of aroma components in Royal Gala apple could differentiate fruits with only small differences in sensory properties. For other apple varieties aroma quality at harvest may have a more dramatic effect on final eating quality.

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