*G. M. SAPERS*
*USDA Eastern Regional Research Center, ARS, Philadelphia, PA 19118*
*and J. ABBOTT, D. MASSIE, A. WATADA and E. E. FINNEY JR.*
*USDA Agricultural Research Center, ARS, Beltsville, MD 20705*

# VOLATILE COMPOSITION OF McINTOSH APPLE JUICE AS A FUNCTION OF MATURITY AND RIPENESS INDICES

———————— ABSTRACT ————————

Research was undertaken to establish relationships between volatile levels in the juice of fresh apples, determined by GLC headspace vapor analysis, and other objective indices of maturity and ripeness. McIntosh apples were tested individually after harvest or following storage and ripening. Volatile levels in the juice were correlated with titratable acidity, Magness-Taylor firmness and stiffness coefficient values. The stiffness coefficient is derived from nondestructive sonic resonance frequency measurements and has potential value for sorting.

## INTRODUCTION

ONE OF THE MAJOR goals of the U.S. apple industry is to improve the quality of apples being marketed. Good quality apples are now widely available throughout the year due to advances in handling and storage practices. However, an excess of poor or low-quality apples are entering marketing channels, and this is a problem of concern to the apple industry. Poor quality fruit may reflect the condition at harvest or may be due to improper storage and handling (Bohall, 1972). Flavor deficiencies constitute an important cause of consumer dissatisfaction with fresh apple quality (Clayton, 1966). One aspect of this problem is the lack of suitable objective methods for the measurement of apple flavor and other attributes of maturity, ripeness and quality (Bohall, 1972; Faust and Kender, 1973).

Extensive research has been carried out on the identification of volatile components of apples. A complex mixture of esters, alcohols and aldehydes contributes to the characteristic apple aroma (MacGregor et al., 1964; Flath et al., 1967, 1969; Picard et al., 1971; Nursten and Woolfe, 1972). A number of investigators have studied the evolution of volatiles by the intact fruit (Meigh, 1957; Grevers and Doesburg, 1965; Brown et al., 1966; Fargas, 1966; Paillard, 1967; Drawert et al., 1969). These studies have related levels of specific volatiles to storage and ripening conditions. However, data comparing volatile formation with other objective indices of maturity or ripeness are generally lacking.

Various chemical and physical properties of apples have been investigated as objective indices of maturity and ripeness. These include measurements of respiration, ethylene, soluble solids, acidity, starch, firmness and color (Market Quality Research Div., ARS, 1965). Nondestructive measurements, e.g., sonic resonance frequency (Abbott et al., 1968; Finney, 1970) and light transmission (Aulenbach et al., 1972), are of particular interest since they can be repeated on individual fruit during maturation and ripening, are compatible with other testing methods and have potential value in automated sorting systems.

Research has been carried out at USDA laboratories in Philadelphia and Beltsville to develop procedures for the measurement of volatile levels in fresh apple juice and to relate such measurements to other objective indices of maturity and ripeness. The results of these studies are reported herein.

## MATERIALS & METHODS

### Materials and experimental design

McIntosh apples were harvested weekly at a commercial orchard in southern Pennsylvania from 3 wk before to 1 wk after normal harvest and at Cornell University in Ithaca, New York, from 1 wk before to 1 wk after normal harvest. All fruit were immediately taken to the Agricultural Research Center in Beltsville, Md., for storage at 0°C and 80–90% relative humidity. Samples were taken for analysis within 1 month and again after 3–4 months' storage followed by 1 wk at 18–20°C for ripening.

A random sample of 20 apples per harvest was analyzed nondestructively for internal pigmentation by measurement of light transmittance (or absorbance, including reflectance and light scattering) between 640 and 880 nm with a high intensity spectrophotometer (Massie and Norris, 1975). The difference in absorbance between 700 and 740 nm ($\Delta$ absorbance) was used as an index of internal chlorophyll. From the original sample, six apples were selected, on the basis of differences in absorbance, external appearance and size, to provide a range of maturity and ripeness.

### Indices of maturity and ripeness

To determine the relationships between volatile composition and other indices of maturity and ripeness, individual apples were evaluated by a number of objective tests prior to volatile analysis. At Beltsville, the apples were analyzed by nondestructive procedures for internal pigmentation as described above. In addition, sonic resonance frequency was measured by the procedure of Finney (1970), and values of the "stiffness coefficient," an index of fruit firmness, were calculated.

After completion of the nondestructive analyses, the apples were shipped by express bus (5 hr portal to portal) to the Eastern Regional Research Center (ERRC) in Philadelphia for further testing. Apples were held at about 1°C and were analyzed within 1 wk. Firmness was measured on peeled portions of the blush and green sides of the apples with a Magness-Taylor pressure tester with a 7/16-in. diameter tip, 5/16-in. penetration, and a 3–30 lb scale. Each apple was immediately cored, sectioned and juiced with an Acme Supreme Juicerator Model 6001 (Acme Juicer Manufacturing Co., Lemoyne, Pa.) lined with Whatman No. 1 filter paper. The juice was analyzed for soluble solids with a Bausch and Lomb Abbe-3L Refractometer and for titratable acidity, expressed as percent malic acid, by diluting 10 ml of juice with 50 ml distilled $H_2O$, and titrating with 0.1N NaOH to a phenolphthalein end point.

### Measurement of volatile levels

Apple volatiles were determined quantitatively by a modification of a GLC headspace vapor procedure developed previously for dehydrated potato products (Sapers et al., 1970, 1972). A 75-ml portion of the juice, extracted from a single apple with the Juicerator, was added to a 250-ml Erlenmeyer flask containing 75 ml distilled $H_2O$, 35.0g NaCl (equivalent to 4M) and a 1-1/2 in. Teflon stirrer bar. The flask was closed with a 16 × 25 mm serum cap, vented with a 3/4 in. #22 gauge hypodermic needle, and was immersed in a constant depth in a 60°C water bath. After 1 min, 1 ml of 300 ppm aqueous methyl acetate (the internal standard) equivalent to 2 ppm in the sample, was added by hypodermic syringe. The sample was equilibrated for 10 min with stirring. Following equilibration, a 2.5 ml vapor sample was withdrawn with a heated gas-tight syringe as described by Sapers et al. (1970).

Vapor samples were analyzed with a Hewlett-Packard Model 7624A gas chromatograph by use of dual 12 ft, 1/8 in. stainless steel columns

containing 15% Carbowax 20M on 80/100 acid washed Chromasorb W, a helium flow rate of 8 ml/min and flame ionization detectors. The injection port and detector were at 200°C, and the column oven was programmed as follows: 60–80°C at 2°/min, 80–120°C at 4°/min, isothermal at 120°C. Retention time and peak area were measured with a Hewlett-Packard Model 3370A Electronic Digital Integrator. Component concentrations were expressed as peak ratios, i.e., the ratios of the component peak areas to the internal standard peak areas.

Conditions for the quantitative analysis of apple volatiles were selected to achieve high sensitivity and precision. NaCl was added to juice samples to increase headspace vapor concentrations of volatile components (Jennings, 1965) and to inhibit enzymatic browning during equilibration (Taüfel and Voigt, 1964). The equilibration time and temperature were chosen to provide high sensitivity without sample degradation as evidenced by chromatographic data and the aroma of the juice after equilibration. Methyl acetate was selected as the internal standard since its retention time coincided with an uncrowded region of apple headspace vapor chromatograms and since its concentration in a number of apple juice samples was found to be low relative to the level of addition. The ratio of endogenous to added methyl acetate was found to be 0.02 for a McIntosh juice and 0.04–0.08 with other samples. In addition, methyl acetate was water soluble at the level of use and was highly stable in aqueous solution and in apple juice.

To estimate the precision of the method, 100 and 300 ml apple juice samples were analyzed in quadruplicate, and peak ratios for the major components were calculated. Mean peak ratios were similar for the two sample volumes. Coefficients of variation for the peak ratios were low (0.9–4.2% for 100 ml samples and 1.0–7.5% for 300 ml samples) and were similar for peaks corresponding to lower and higher boiling components.

Authentic samples were obtained of all compounds which had been reported previously in apple volatiles and had been identified by retention time and mass spectral or IR analysis. Retention times of these compounds were determined under the GLC conditions described above to tentatively identify the headspace vapor components of apple juice samples.

### Statistical analyses

Population means, standard deviations, correlation coefficients and their confidence intervals (Steel and Torrie, 1960) were computed for the measurements obtained with fresh apples and their juice. In addition, relationships between parameters were displayed graphically.

## RESULTS & DISCUSSION

### GLC headspace vapor analyses

GLC headspace vapor analyses of fresh apple juice samples (Fig. 1) revealed the presence of a complex mixture of volatile components. Previously reported apple volatiles which have retention times similar to those of the major headspace vapor components of McIntosh apple juice are listed in Table 1.

Headspace vapor analyses of fresh McIntosh apple juice yielded a characteristic pattern of peaks. Quantitative differences in volatile composition between individual apples were large, presumably due to differences in fruit quality, condition and ripeness. Typical chromatographic data for apples which were harvested on different dates and varied in ripeness are summarized in Table 2. Juice from apples having the characteristic aroma of the ripe fruit contained elevated levels of peaks 13, 18, 19, 21, 25, 27 and 30, and variable amounts of peaks 6, 10 and 12; the latter three peaks tended to increase during storage. These results are generally similar to the data reported by Fargas (1966) for headspace gas above ripening McIntosh apples. He tentatively identified (by retention time) the following major components: ethanol, ethyl propionate, ethyl butyrate, n-propanol, 2-methylpropanol, 3-pentanol, 2-pentanol, n-butanol, 3-methylbutanol, ethyl hexanoate and n-hexanol. MacGregor et al. (1964) identified 2-methylpropanol, n-butanol, 2-methylbutanol, 3-methylbutanol, n-pentanol, n-hexanol, hexanal, 2-hexenal, 3-methylbutyl acetate, ethyl butyrate and ethyl hexanoate in McIntosh apple juice by retention time and IR analysis.

In examining the relationship between volatile levels and indices of maturity and ripeness, we considered the characteristic McIntosh peaks individually. We also computed the sums of the peak ratios for peaks 13, 18, 19, 21, 22, 25, 27 and 30 which are consistently high in the ripe fruit.

**Table 1—Retention times of potential McIntosh apple headspace vapor components**

| Peak no. | Compound | Retention time (min) Apple juice | Ref. cpd. | References[a] |
|---|---|---|---|---|
| 6 | Acetaldehyde | 3.79 | 3.72 | 1,2 |
| 10 | Ethyl acetate | 8.07 | 7.91 | 1,2 |
| | 1-Butanal | 7.99 | 7.86 | 1,2 |
| 12 | Ethanol | 9.09 | 9.19 | 1,2 |
| | 2-Methylbutanal | 9.09 | 9.19 | 1,2 |
| | 3-Methylbutanal | 9.09 | 9.38 | 1,2 |
| 13 | Ethyl propionate | 10.91 | 10.72 | 1,2,3 |
| | Ethyl 2-methylpropionate | 10.79 | 11.14 | 1,2 |
| 18 | Ethyl butyrate | 14.37 | 14.33 | 1,2,3,4 |
| | 1-Propyl propionate | 14.29 | 14.61 | 1 |
| 19 | Ethyl 2-methylbutyrate | 14.78 | 14.78 | 1,2,3 |
| 21 | Hexanal | 16.59 | 16.69 | 1,2,3,4 |
| 22 | 1-Butanol | 17.98 | 18.19 | 1,2,3,4 |
| | 3-Methylbutyl acetate | 18.07 | 18.08 | 2,4 |
| | 2-Methylbutyl acetate | 17.82 | 17.68 | 1,2 |
| | 1-Propyl butyrate | 17.82 | 17.78 | 1,2,3 |
| | Ethyl pentanoate | 18.05 | 18.52 | 1,2 |
| | Amyl acetate | 18.29 | 18.03 | 1 |
| 25 | 2-Methylbutanol | 20.80 | 20.85 | 1,2,3,4 |
| | 3-Methylbutanol | 20.77 | 20.78 | 2,4 |
| | 2-Heptanone | 20.86 | 20.67 | 2 |
| 27 | trans-2-Hexenal | 22.98 | 22.73 | 1,2,3,4 |
| | Ethyl hexanoate | 23.06 | 22.97 | 1,2,4 |
| 30 | Hexanol | 29.75 | 29.41 | 1,2,3,4 |

[a] 1 = Flath et al. (1967); 2 = Flath et al. (1969); 3 = Nursten and Woolfe (1972); 4 = MacGregor et al. (1964).

**Table 2—Levels of headspace vapor components and ripeness indices for individual McIntosh apples**

| Peak no. | Peak ratio — Harvest date 8/19 | 9/13 | 9/16 | 9/16[a] |
|---|---|---|---|---|
| 6 | 0.00 | 0.03 | 0.08 | 0.40 |
| 10 | 0.01 | 0.02 | 0.06 | 0.14 |
| 12 | 0.00 | 0.16 | 0.90 | 2.38 |
| 13 | 0.02 | 0.41 | 0.63 | 1.10 |
| 18 | 0.00 | 0.89 | 2.91 | 3.30 |
| 19 | 0.00 | 0.23 | 0.54 | 0.82 |
| 21 | 0.04 | 0.20 | 0.74 | 0.66 |
| 22 | 0.07 | 0.66 | 1.88 | 1.73 |
| 25 | 0.02 | 0.54 | 1.06 | 0.87 |
| 27 | 0.03 | 0.18 | 0.52 | 0.52 |
| 30 | 0.17 | 0.84 | 2.00 | 1.50 |
| Sum[b] | 0.35 | 3.95 | 10.28 | 10.50 |
| Titratable acidity (%) | 1.40 | 1.15 | 0.59 | 0.58 |
| Soluble solids (%) | 11.2 | 14.5 | 13.7 | 13.3 |
| Magness-Taylor, green (lb) | 15.5 | 11.7 | 7.2 | 7.0 |

[a] Stored 4 months
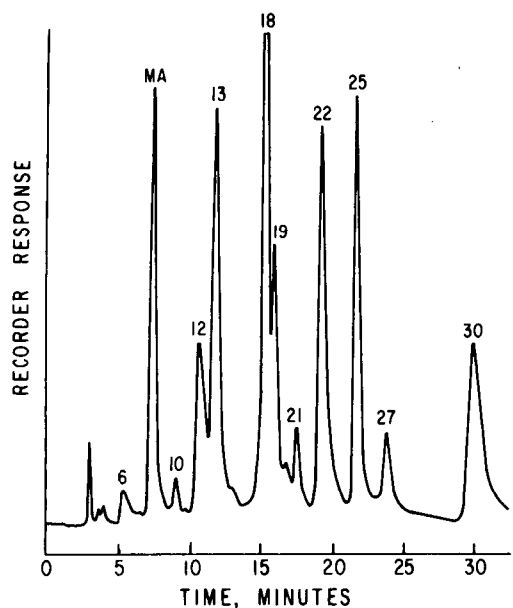[b] Sum of peak ratios for peaks 13, 18, 19, 21, 22, 25, 27, 30

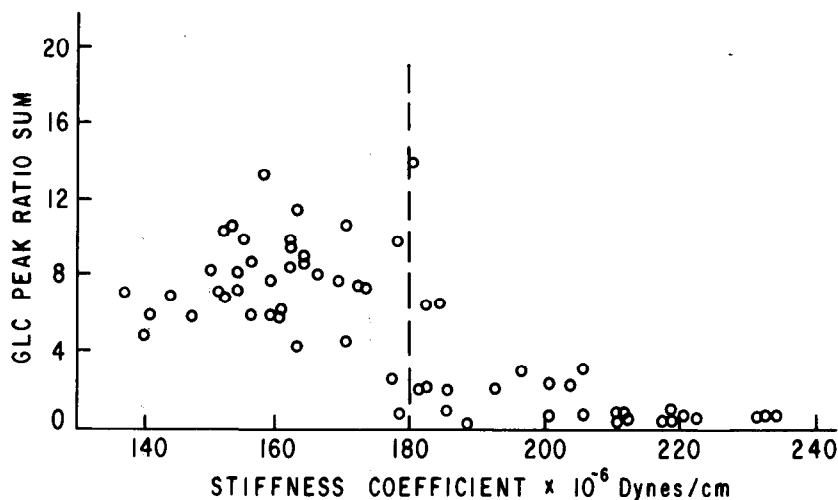Fig. 1–Headspace vapor chromatogram for ripe McIntosh apple juice sample.



Fig. 2–Volatile level vs stiffness coefficient in McIntosh apples.

Table 3—Mean volatile levels and maturity indices in McIntosh apples

| Parameter | Pennsylvania McIntosh | | New York McIntosh | |
|---|---|---|---|---|
| | Stored 1 month[a] | Stored 4 months and ripened[b] | Stored 1 month[a] | Stored 4 months and ripened[b] |
| GLC peak ratio sum | 3.06 | 8.66 | 0.76 | 6.13 |
| Acidity (%) | 1.10 | 0.70 | 1.34 | 0.86 |
| Soluble solids (%) | 12.7 | 12.3 | 12.8 | 12.4 |
| Magness-Taylor, red (lb) | 13.2 | 8.5 | 12.2 | 9.0 |
| Magness-Taylor, green (lb) | 11.9 | 8.4 | 12.7 | 8.6 |
| Δ Absorbance | 0.76 | 0.78 | 0.80 | 0.84 |
| Stiffness coeff. (dynes/cm X $10^{-6}$) | 181 | 160 | 211 | 159 |
| Sample size | 31 | 17 | 18 | 16 |

[a] Stored at 0°C and 80—90% RH after harvest
[b] Stored at 0°C and 80—90% RH after harvest; then held for 1 wk at 18—20°C for ripening.

## Correlation of volatile levels with maturity and ripeness indices

Relationships between volatile levels and the other objective indices were evaluated by statistical analysis of data for individual apples from the different populations in this study. Mean values of these parameters are summarized in Table 3. Harvest date was not included as a variable in the analysis because of the limited sample size, and data corresponding to the different harvest dates were pooled. In McIntosh apples ripened at 18—20°C after 4 months' storage at 0°C, volatile levels were higher while titratable acidity, Magness-Taylor firmness and stiffness coefficient values were lower than in fruit stored for 1 month at 0°C. Soluble solids were relatively constant. Absorbance difference values were highly variable and showed no trend.

Correlations between volatile levels and objective indices of maturity and ripeness are generally low in the relatively homogeneous populations of McIntosh apples from the different storage periods considered separately (Table 4). The Pennsylvania McIntosh stored for 1 month were more heterogeneous than the New York McIntosh, and consequently, correlation coefficients for these apples were higher. Correlation coeffi-

Table 4—Correlation coefficient between volatile levels and indices of maturity and ripeness in different populations of McIntosh apples

| Volatile level[a] vs | Pennsylvania | | | New York | | | Pennsylvania and New York Pooled |
|---|---|---|---|---|---|---|---|
| | Stored 1 month | Stored 4 months and ripened | Pooled | Stored 1 month | Stored 4 months and ripened | Pooled | |
| Acidity | −0.74 | −0.35 | −0.83 | −0.44 | 0.26 | −0.90 | −0.85 |
| Magness-Taylor (green) | −0.69 | −0.05 | −0.77 | −0.67 | 0.58 | −0.84 | −0.77 |
| Stiffness Coefficient | −0.64 | 0.28 | −0.53 | −0.13 | 0.15 | −0.84 | −0.72 |

[a] GLC peak ratio sum

cients for the pooled data, representing a wide range of ripeness, were higher and show potentially useful relationships between volatile levels and acidity, Magness-Taylor firmness and the stiffness coefficient (Tables 5 and 6). In general, the peak ratio sum was more highly correlated with maturity and ripeness indices than were the individual peak ratios. Confidence intervals for correlation coefficients at the 95% level showed that these relationships are highly correlated. Correlations between soluble solids, absorbance difference values and the other parameters were not significant.

The practical value of these correlations is indicated in the graphical presentation of the peak ratio sum and stiffness coefficient data for all McIntosh apples in the study (Fig. 2). Apples having low or high volatile levels can be distinguished on the basis of the stiffness coefficient (i.e., above or below 180 $\times 10^6$ dynes/cm). The value of the peak area ratio sum within

these categories, however, cannot be predicted accurately. Apples classified by sonic resonance frequency will differ in firmness and acidity as well as in volatile level since the former factors are also correlated with the stiffness coefficient. If these relationships were generally true with McIntosh or other varieties of apples, they might be sorted according to volatile level (and ripeness) on the basis of a nondestructive test. Research to this end is continuing.

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Reference to a brand or firm name does not constitute endorsement by the U.S. Dept. of Agriculture over others of a similar nature not mentioned.

Table 5—Correlation between volatile levels and indices of maturity and ripeness in pooled McIntosh apples

| GLC Peak | Acidity | Magness-Taylor (green) | Stiffness coefficient | Soluble solids | Δ Absorbance |
|---|---|---|---|---|---|
| 6 | −0.72 | −0.57 | −0.69 | −0.42 | −0.01 |
| 10 | −0.68 | −0.60 | −0.67 | −0.36 | −0.08 |
| 12 | −0.76 | −0.62 | −0.71 | −0.37 | −0.08 |
| 13 | −0.79 | −0.69 | −0.79 | −0.26 | −0.20 |
| 18 | −0.79 | −0.70 | −0.68 | −0.12 | −0.33 |
| 19 | −0.76 | −0.63 | −0.64 | −0.21 | −0.29 |
| 21 | −0.72 | −0.72 | −0.60 | 0.09 | −0.34 |
| 22 | −0.79 | −0.78 | −0.59 | 0.03 | −0.53 |
| 25 | −0.68 | −0.68 | −0.63 | 0.15 | −0.53 |
| 27 | −0.78 | −0.66 | −0.62 | −0.11 | −0.37 |
| 30 | −0.72 | −0.69 | −0.47 | 0.10 | −0.53 |
| Peak ratio sum | −0.85 | −0.77 | −0.72 | −0.09 | −0.40 |
| High[a] | −0.90 | −0.84 | −0.81 | −0.30 | −0.56 |
| Low[a] | −0.78 | −0.66 | −0.60 | 0.13 | −0.20 |

[a] Confidence interval for correlation coefficient at 95% level

Table 6—Correlations between indices of maturity and ripeness in pooled McIntosh apples

| Comparison | Correlation coefficient | Confidence interval[a] Low | Confidence interval[a] High |
|---|---|---|---|
| Acidity vs Stiffness coefficient | 0.82 | 0.74 | 0.88 |
| Magness-Taylor (green) vs Acidity | 0.81 | 0.73 | 0.88 |
| Stiffness coefficient | 0.72 | 0.60 | 0.81 |

[a] 95% level