Identification and Organoleptic Evaluation of Compounds in Delicious Apple Essence

R. A. FLATH, D. R. BLACK, D. G. GUADAGNI, W. H. MCFADDEN, AND T. H. SCHULTZ

Commercial Delicious apple essence was extracted to yield an oil with a strong apple-like aroma. This oil was separated into its components with high-resolution packed and open-tubular gas chromatographic columns. Fifty-six compounds were identified, mainly by a combination of mass spectrometry and gas-chromatographic retention data. While many of these compounds contribute to over-all Delicious apple essence aroma, the main components directly associated with characteristic apple-like aroma were ethyl 2-methylbutyrate, hexanal, and 2-hexenal.

Identification of volatile apple constituents has been a subject of considerable interest to investigators for nearly 50 years (*23*). However, progress in this area has been hindered by two problems familiar in aroma research: Appreciable quantities of apple volatiles were difficult to obtain from fresh fruit, and the analytical techniques were tedious and insensitive to trace components and were particularly unsatisfactory for ester identification. The development of an efficient essence-recovery unit (*18*), which provides a means of concentrating most of the volatiles in apple juice by 100 to 150 times, and the use of cold traps and/or activated carbon beds for removal of volatiles from apple storage chamber atmospheres provided two fairly satisfactory alternative approaches to the concentration of volatiles (*3, 4, 7, 8, 12, 15, 17, 21, 22, 28, 29, 33–36*). Difficulties encountered in separation and identification of the individual constituents were partially resolved by the application of gas chromatographic (GC) techniques. This technique greatly facilitates rapid separation of apple volatile mixtures, but by its nature does not provide much information for identification. Retention times are often employed for this purpose, but especially with the short, low-retention packed columns commonly in use, this method of identification is unreliable. Recognizing this limitation, a number of workers have devised reaction sequences and additional separation techniques to be used in conjunction with GC (*2, 21, 33*). The combination of high-resolution, open-tubular columns with a fast-scan mass spectrometer has proved to be of great value in helping to provide unambiguous identifications for the separated components of fruit volatiles (*13, 27*).

A list of reported apple volatiles, taken directly from the literature without any attempt to evaluate them, is given in Table I. The bases for the identifications vary from the careful separation and chemical work of such investigators as White (*36*), Strackenbrock (*28*), and Nishimura and Hirose (*21*), to a complete absence of any supporting data. A considerable number of these compounds have been reported to be major contributors to the characteristic aroma of apples. Power and Chesnut (*23*) felt that the amyl esters of formic, acetic, caproic, and caprylic acids were important, along with acetaldehyde, and that odor differences among apple varieties might be the result of variations in the relative proportions of these compounds. The C_3 to C_6 alcohols and their esters were claimed by Kieser and Pollard (*10*) to be essential. Strackenbrock (*28*) pointed out the importance of butyl and hexyl acetates. White (*36*) first found 2-hexenal in apple essence, and Koch and Schiller (*12*) reported it to be an essential constituent of apple aroma. Unfortunately, most workers have not employed judging panels for organoleptic evaluation, so such judgments are difficult to evaluate, particularly when the apple variety employed differs from study to study.

Previous work on Delicious apple essence by Guadagni *et al.* (*6*) showed that extract from the essence could be separated by GC into fractions possessing characteristic apple aroma. Because this early work was conducted with short packed columns (Apiezon L), resolution was relatively poor, and the authors recognized that the odorous fractions were probably mixtures rather than single components. Therefore, perhaps the apple-like aromas of various fractions were due to mixtures of several compounds, and these compounds, once isolated, might not exhibit apple-like aromas individually. Therefore, one of the objectives of the organoleptic work reported here was to determine whether the aromas of some individual bands eluted from the high-resolution open-tubular GC columns might still be considered apple-like.

Experimental

Equipment. All GC equipment used in this study was made in the authors' laboratories. For judging the aroma of individual bands, open-tubular columns, 500 and 1000 feet in length, 0.03-inch I.D. (*31*), coated with methyl silicone oil SF-96(50) containing 5% Igepal (nonyl phenoxypolyoxyethylene ethanol) (*19*), were used in conjunction with a nondestructive microthermistor detector. For mass spectral analyses with the Bendix

Western Regional Research Laboratory, Western Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Albany, Calif.

Table I. Reported Apple Volatiles—1920–66

Free and Esterified Alcohols	References
Methanol	(*7, 9, 10, 12, 16, 21, 23, 26, 27, 30, 35*)
Ethanol	(*2, 7, 10, 11, 14, 16, 17, 21, 22, 23, 25, 28, 29, 36*)
Propanol	(*7, 10, 11, 14, 16, 21, 22, 25, 28, 29, 36*)
2-Propanol	(*2, 7, 10, 12, 14, 17, 21, 29, 36*)
Butanol	(*2, 7, 10, 13, 14, 16, 21, 22, 25, 28, 29, 36*)
2-Methylpropan-1-ol	(*2, 10, 12, 13, 14, 17, 21, 22, 29, 36*)
Pentanol	(*2, 7, 10, 12, 13, 14, 16, 21, 22, 23, 28, 29, 35*)
2-Methylbutan-1-ol	(*14, 17, 21, 25, 28, 29, 35, 36*)
3-Methylbutan-1-ol	(*2, 10, 11, 14, 25, 28, 29*)
Hexanol	(*2, 7, 10, 12, 13, 14, 16, 17, 21, 25, 28, 29, 36*)
"*n*-Hexenol"	(*25*)
trans-2-Hexen-1-ol	(*1*)
3-Hexen-1-ol	(*21*)
Geraniol	(*24*)
Esters	
Methyl formate	(*13, 14, 29*)
Ethyl formate	(*2, 14, 29*)
Methyl acetate	(*2, 10, 11, 25, 28*)
Ethyl acetate	(*2, 10, 11, 13, 14, 22, 25, 28, 29*)
2-Propyl acetate	(*10*)
Propyl acetate	(*10, 21, 22*)
2-Methylpropyl acetate	(*10*)
Butyl acetate	(*10, 14, 21, 22, 25, 28, 29*)
3-Methylbutyl acetate	(*10, 14, 28, 29*)
Pentyl acetate	(*13, 14, 21, 22, 25, 28, 29*)
Hexyl acetate	(*21, 28*)
Ethyl propionate	(*11, 22, 25, 28*)
2-Methylpropyl propionate	(*14, 29*)
Butyl propionate	(*22*)
Hexyl propionate	(*28*)
Methyl butyrate	(*10*)
Ethyl butyrate	(*10, 13, 14, 22, 25, 29*)
Propyl butyrate	(*10, 22, 29*)
Butyl butyrate	(*10, 22, 28*)
Pentyl butyrate	(*28*)

Esters	References
Ethyl pentanoate	(*22, 28*)
Propyl pentanoate	(*22*)
Methyl 3-methyl butyrate	(*28*)
3-Methylbutyl 3-methyl butyrate	(*4*)
Methyl hexanoate	(*14, 29*)
Ethyl hexanoate	(*10, 14, 21, 28*)
Butyl hexanoate	(*28*)
Ethyl octanoate	(*4*)
Free and esterified acids	
Formic	(*7, 11, 14, 16, 17, 23, 32, 35, 36*)
Acetic	(*7, 11, 14, 16, 17, 22, 28, 32, 35, 36*)
Propionic	(*7, 11, 14, 16, 17, 22, 28, 32, 36*)
2-Methylpropionic	(*12*)
Butyric	(*7, 12, 16, 17, 22, 28, 36*)
3-Methylbutyric	(*12, 21, 28*)
Pentanoic	(*7, 17, 22, 28, 32*)
4-Methylpentanoic	(*12*)
Hexanoic	(*7, 12, 14, 17, 21, 23, 28, 32, 36*)
Octanoic	(*12, 16, 23*)
"*n*-Hexenoic"	(*12*)
Benzoic	(*21*)
Carbonyls	
Formaldehyde	(*11, 13, 21*)
Acetaldehyde	(*7, 9, 10, 11, 14, 16, 17, 21, 22, 23, 29, 36*)
Propanal	(*7, 9, 10, 11, 14, 17, 21, 29*)
Butanal	(*10, 12, 17, 21*)
2-Methylpropanal	(*17*)
Pentanal	(*10, 12*)
3-Methylbutanal	(*17*)
Hexanal	(*12, 14, 22, 28, 36*)
2-Hexenal	(*12, 14, 21, 25, 36*)
Nonanal	(*21*)
Furfural	(*36*)
Acetone	(*7, 9, 12, 14, 16, 17, 22, 29, 35, 36*)
2-Butanone	(*12, 17*)
2-Pentanone	(*17*)
2-Hexanone	(*10*)
Acetophenone	(*16*)
Diacetyl	(*10*)

Model 12, Time-of-Flight mass spectrometer, open-tubular columns, 500 feet, 0.02-inch I.D., coated like the 0.03-inch I.D. columns, were used. A hydrogen flame ionization detector was used to check retention times.

Components which could not be identified directly by their mass spectra were isolated by fractional distillation with a 61 × 0.8 cm. platinum spinning band column and by packed column preparative GC. Packed columns used were: 18 feet, 1-inch O.D., packed with 70- to 80-mesh silanized Chromosorb G impregnated with 5% methyl silicone oil SF-96(50) plus 0.1% Igepal; 30 feet, $^5/_8$-inch O.D., packed with 70- to 80-mesh Chromosorb G impregnated with 5% Carbowax 20M (polyethylene glycol); and 100 feet, $^1/_4$-inch O.D., packed with 100- to 120-mesh Chromosorb G impregnated with 1% Carbowax 20M.

Extraction. Commercial 150-fold Delicious apple essence (Diamond Fruit Growers, Hood River, Ore.) was used as a source of apple volatiles in this study. Three different solvent systems were used for extraction of separate batches of essence, but in all three runs, the same apparatus (Figure 1), constructed entirely of glass, stainless steel, and Teflon, was used. The essence flow rate through the countercurrent extraction unit was approximately 450 ml. per minute, with the solvent flow rate $^1/_{10}$ to $^1/_4$ of the essence rate.

Isopentane. A total of 288 liters (76 gallons) of essence was extracted with purified 2-methylbutane (isopentane, 28.8 liters, 7.6 gallons, Phillips 99% distilled through a 10-plate Oldershaw column, then filtered through a 2-meter × 15-cm. column of Alcoa F-20 alumina). The bulk of the isopentane was distilled from the extract through the Oldershaw column (maximum head temperature, 28° C.), and the remaining solvent was removed with a 32 × 1.8 cm. glass-helix-packed distillation column. A maximum water bath temperature of 55° C. and head temperature of 28° C. were attained. A light yellow oil, still containing traces of solvent (by GC), was obtained (101.2 grams).

Ether. A total of 106 liters (28 gallons) of essence was extracted with 26.5 liters (7 gallons) of ether (Baker analyzed anhydrous reagent grade). The extract was dried with anhydrous sodium sulfate, and the solvent was removed by the same sequence as above. A maximum bath temperature of 68° C. and a head temperature of 37° C. were reached. A yellow oil (160.8 grams), containing a trace of solvent, was obtained.

Ether–Isopentane. A total of 106 liters (28 gallons) of essence was extracted with 22.7 liters (6 gallons) of 1:1 ether–isopentane. After workup, as in the ether extraction (bath T = 70° C., head T = 37° C.), 152.0 grams of liquid remained, containing traces of solvent.

Analytical Procedure

High-resolution open-tubular columns, 500 feet, 0.02-inch I.D., coated with SF-96 (50) containing 5% Igepal, were used for examination of the apple extracts. For preliminary analyses the isopentane extract was chosen, because of the lower concentration of low molecular weight alcohols in this material as compared with the other extracts (*26*). The lower alcohols tend to tail on the SF-96 (50) columns, even with the addition of Igepal (*19*), and this tailing complicates mass spectral interpretations. The open-tubular column was connected directly to the ionization chamber of the mass spectrometer. Operation of the open-tubular GC column–mass spectrometer combination has been described (*13*, *27*). The column oven temperature was held at 50° C. for 10 minutes after injection of approximately 0.1 μl. of sample, then was programmed at 1° C. per minute for 110 minutes, followed by 10 minutes at 3.5° C. per minute. The pressure at the injector end of the column was set at approximately 22 p.s.i. absolute (7 p.s.i. above atmospheric pressure). This pressure provided a linear velocity of 24 cm. per second at 50° C. A typical chromatogram, using a hydrogen-flame detector, is shown in Figure 2.

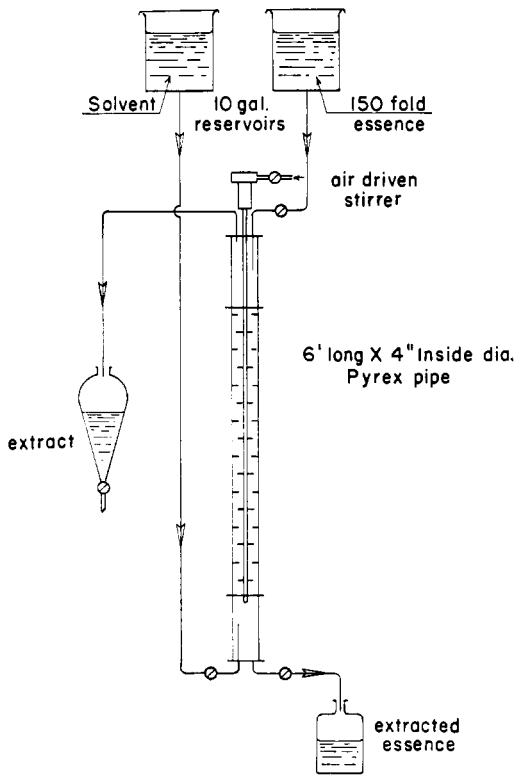

Figure 1. Countercurrent essence extractor

Mass spectral assignments for the chromatogram peaks were checked by co-injection of authentic samples (either purchased or synthesized) along with a sample of the entire extract. An increase in the peak's size relative to the rest of the chromatogram was taken as verification of the identification. Components which were isolated by fractional distillation and preparative GC were identified by various combinations of infrared, nuclear magnetic resonance, mass spectrometry, and retention time data.

Organoleptic Evaluation

The procedure used for odor description assignments to the various peaks obtained from apple essence has been described (*6*). Briefly, the column effluent was sniffed by judges trained in apple odor evaluation. The judges described each distinct odor as it emerged from the thermistor detector, and an attendant at a remotely positioned recorder noted the description on the chromatogram as it was recorded. The entire procedure was repeated at least three times by each judge. In the present study, five judges were employed. A peak was considered to have sensory significance when 80% or more of the judgments described the effluent as having an apple-like aroma. For a chromatogram with a minimum of 10 separate and distinct odors, the chance of selecting any given odor as being apple or apple-like by guessing alone is one tenth, or 10%. Therefore, the probability that 80%, or 12 of the 15 trials, would result in selection of the same peak as representing an apple or apple-like odor by chance would be less than 10^{-7} (*20*). Thus the authors felt that this degree of concurrence on

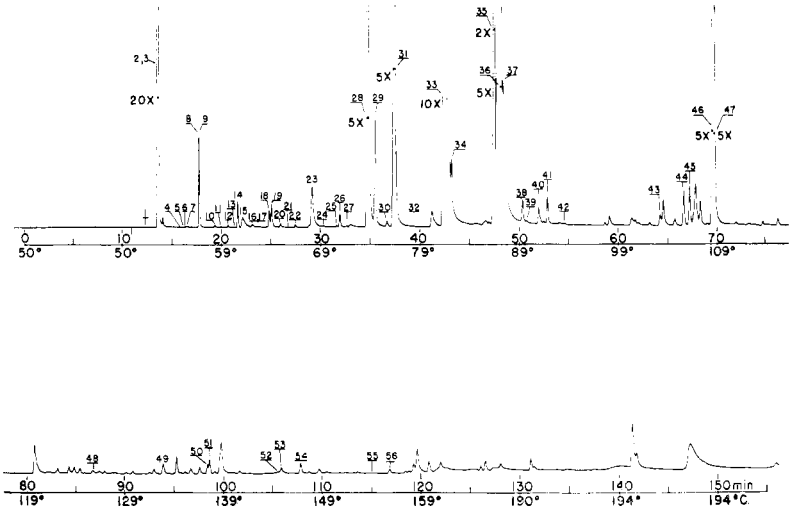

Figure 2. Chromatogram of isopentane extract—0.04 μl. injected on a 500-foot × 0.02-inch I.D. open-tubular column coated with SF-96(50) containing 5% Igepal

assignment of apple odor to a specific peak represents a high degree of sensory significance.

The relative odor intensities of the compounds corresponding to the peaks selected by the procedure described above, as well as those of a number of other components of apple essence extract, were estimated by odor threshold measurements. All compounds were purified by GC, and their threshold concentrations were determined within 2 hours after purification. Except for substitution of Teflon for polyethylene squeeze bottles as the odor solution reservoir and delivery system, threshold measurements were conducted as previously described (*5*). The panel consisted of 20 to 26 judges who were screened from a pool of 60 people for olfactory acuity to the compounds found in apple essence. The threshold was taken as that concentration at which the full panel was able to distinguish the compound from triple-distilled water at $P \leqslant 0.01$.

All odor measurements were conducted in individual booths which were maintained at 21.1° C. and were swept by a stream of air purified by passage through activated charcoal. The air flow was regulated to maintain a slight positive pressure in the panel room to minimize the entrance of extraneous odors.

Results and Discussion

Compound identifications are given in Table II. The isopentane solvent used in the extraction contains components 3, 5, 7, 9, 14, and 25, so these compounds are not thought to be present in the apple essence. They were not detected in ether-extracted material.

The various acetals identified may be artifacts; they were found to be present in higher concentration in year-old essence than in relatively fresh essence.

No 3-methylbutan-1-ol, 3-methylbutyl esters, or 3-methyl butyrate esters were detected during the mass spectrometry–GC runs, although a small amount of 3-methylbutanal was found. In contrast, 2-methylbutan-1-ol, 2-methylbutyl acetate, and several 2-methyl butyrate esters were identified. The column employed is capable of resolving the 2- and 3-methylbutan-1-ols as well as the 2- and 3-methylbutyl acetates, so any appreciable concentrations of the 3-methyl isomers would likely have been detected.

In the extractions, no attempt was made to extract the essence completely. However, the yields reported here are similar to those reported by Schultz *et al.* (*26*), who investigated the yields from samples of the same essence used in this study, using a batch technique with four successive extractions.

The solvent stripping procedure outlined removed considerable amounts of the more volatile compounds such as acetaldehyde with the solvent. Such losses were not considered a significant drawback, for the authors' interest was directed primarily toward the identification of the higher-boiling compounds, some of which exhibited the desired odor qualities.

A total of over 400 grams of yellow oil with a strong apple-like odor was obtained. This supply provided more than enough material for distillation, preparative GC, and other separation procedures still planned.

High-resolution open-tubular GC columns helped considerably in separating the components of the extracted oil. The separative power of such columns is illustrated by Figure 2. The lower-capacity 0.02-inch I.D. columns were used for delivering the separated components of the extracts directly to the mass spectrometer, while the larger-capacity 0.03-inch I.D. columns, which provided nearly as much resolution as 0.02-inch I.D. columns of the same length, were used with thermistor detectors for odor descriptions of components of the various fractions.

Substitution of an open-tubular column for a packed column provides no guarantee that the eluted bands, or peaks, consist of only one compound; in fact, in a number of cases several constituents were eluted simultaneously. However, most of the constituents were well separated, and this high resolution greatly simplified the selection of significant aroma-contributing compo-

Table II. Delicious Apple Essence Extract Components

1	Acetaldehyde	29	Ethyl butyrate
2	Ethanol	30	Propyl propionate
3	Isopentane (2-methyl-butane)[a]	31	Butyl acetate
4	Propanol	32	1-Ethoxy-1-propoxy-ethane[b]
5	2-Methylpentane[a]	33	*trans*-2-Hexenal (infrared)
6	Butanal	34	Ethyl 2-methyl butyrate[b]
7	3-Methylpentane[a]	35	2-Methylbutyl acetate[b]
8	Ethyl acetate	36	Hexanol (infrared, mass spectrometry, retention time)
9	Hexane[a]	37	*trans*-2-Hexen-1-ol (infrared, NMR, mass spectrometry)
10	2-Methylpropan-1-ol	38	Propyl butyrate
11	3-Methylbutanal	39	Ethyl pentanoate
12	2-Methylbutanal[b]	40	Butyl propionate
13	1-Ethoxy-1-methoxy-ethane[b]	41	Pentyl acetate
14	Cyclohexane[a]	42	1-Butoxy-1-ethoxy-ethane[b]
15	Butanol	43	1-Ethoxy-1-(2-methyl-butoxy)ethane[b]
16	Pentanal	44	Butyl butyrate
17	3-Pentanone[b]	45	Ethyl hexanoate
18	Ethyl propionate	46	Hexyl acetate
19	Propyl acetate	47	*trans*-2-Hexen-1-yl acetate[b] (infrared, NMR)
20	Methyl butyrate	48	1-Ethoxy-1-hexoxy-ethane[b]
21	2,4,5-Trimethyl-1, 3-dioxolane[b]	49	Benzyl acetate[b]
22	1,1-Diethoxyethane[b]	50	Butyl hexanoate
23	2-Methylbutan-1-ol	51	Hexyl butyrate[b]
24	Ethyl 2-methyl propionate[b]	52	Ethyl 2-phenyl acetate[b]
25	Toluene[a]	53	Pentyl 2-methyl butyrate[b]
26	2-Methylpropyl acetate	54	2-Phenylethyl acetate[b]
27	Methyl 2-methyl butyrate	55	2-Methylnaphthalene[b]
28	Hexanal	56	1-Methylnaphthalene[b]

[a] Probably from solvent.
[b] Previously unreported in apple volatiles.

nents in apple essence extracts. Certainly, without such columns and without preliminary separation and identification, studies such as that conducted with the isomeric pentyl acetates (*30*), the separation and identification of ethyl 2-methyl butyrate, found to be important in apple essence aroma, would not have been possible.

Odor evaluation of the effluent from the open-tubular columns showed consistently that material represented

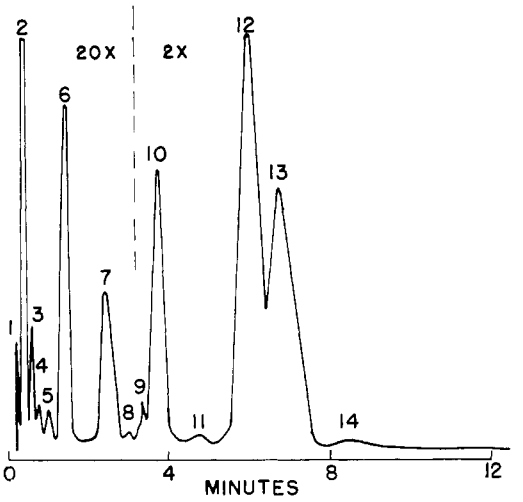

Figure 3. Chromatogram of apple essence extract on 15-foot × 1/8-inch O.D. 15% Apiezon L–packed column

by peaks 28, 33, and 34 (Figure 2) received apple or apple-like descriptions. In 15 trials, these peaks were characterized as good, green, or ripe apple in 13 (over 85%) of the evaluation runs. Many of the other peaks were described as pleasant, fruity, or ester-like, but not as having any direct resemblance to a characteristic apple aroma.

In order to determine which, if any, of the peaks from the open-tubular columns correspond to the apple fraction peaks obtained from packed columns, effluent vapor (5 ml.) corresponding to each apple fraction or peak of the previous study (*6*) from a packed column was collected and injected into an open tubular column. Table III shows that peak 10 from the packed column contains peaks 28, 30, and 31 (Figures 2 and 3) from the open-tubular column. Peak 11 from the packed column is apparently homogeneous, and peak 12 from the packed column was found to contain compounds corresponding to peaks 33, 35, and 36 from the open-tubular column. At least one of the peaks resolved from each of the packed column peaks with the open-tubular column was still characterized as apple or apple-like by the odor judges, who sniffed the effluent from the open-tubular column. Thus, a complex mixture apparently is not essential for perception of an apple-like aroma. On the other hand, it is possible that the effluent corresponding to the individual, apparently well-resolved peaks contains enough "tail" from previous peaks to give the observed odor. However, some of the peaks—e.g., 29, 30, 31, and 36, Figures 2 and 3—found in the packed column fractions are not characterized as apple-like. Whatever the mechanism of the olfactory perception of apple aroma may be, these results indicate that the same characteristic apple-like odor was obtained from both a packed and an open-tubular column. Furthermore, the characteristic odors were observed at the retention times for those compounds which correspond to peaks 28, 33, and 34 (Figure 3) on both types of column.

Table III. Assignment of Apple-like Character to Column Effluent Peaks

Apiezon L-Packed Column		SF-96(50) Open-Tubular Column	
Peak	Odor description	Peak(s)	Peak with apple-like odor
10	Green apple	28, 30, 31	28
11	Ripe apple	34	34
12	Green apple	33, 35, 36	33

Threshold measurements of purified samples of some of the compounds found in the apple essence extracts are shown in Table IV. As expected, the alcohols had the highest thresholds, and the esters and aldehydes the lowest. Ethyl 2-methyl butyrate, a previously unreported compound in apple volatiles, had the lowest threshold of the compounds characterized as having an apple or apple-like aroma. This confirms the previous finding that peak 11 (*6*) from the packed column had the most intense odor and was described as having a ripe or overripe Delicious apple odor. Hexanal exhibited the next lowest threshold and was characterized as having a green apple odor. This compound is eluted as part of peak 10 from the packed column. Peak 10 was considered to have the second most intense apple aroma in the earlier work, but the intensity was determined to be considerably lower than that of peak 11. Again, this agrees with the threshold odor intensity of pure hexanal. The least intense odor (highest threshold) of the compounds directly associated with apple-like aroma was attributed to 2-hexenal. Thus the relative order of odor intensities determined from the packed column peaks ($11 > 10 > 12$) is the same as the order determined by threshold concentration for the apple-like compounds found by open-tubular column analyses to be present in those packed column peaks—ethyl 2-methyl butyrate, hexanal, and 2-hexenal, with thresholds of 0.1, 5, and 17 p.p.b., respectively.

Certainly, many of the other compounds identified and listed in Table II make important contributions to the over-all apple aroma (as may some not yet identified), particularly the esters, with their relatively low thresholds. Another factor which must be considered in attempting to determine the relative importance of the various compounds present to the over-all apple aroma is the concentration of the individual components in fresh fruit. Much further work with fresh apples is necessary before any assessment of this factor can be made. Commercial essence, while easily available, is commonly made in large part from peels and cores, and so provides a somewhat erroneous view of the relative concentrations of the compounds in fresh fruit. The results reported here are applicable in detail only to the particular commercial essence extracted. Some of the compounds, such as the mixed acetals, may be artifacts not present in freshly picked fruit.

Table IV. Olfactory Thresholds of Compounds Identified in Delicious Apple Essence Extracts

	Compound	Threshold, P.P.M. (V./V.)
Alcohols	Ethanol	100
	Propanol	9
	Butanol	0.5
	Hexanol	0.5
Aldehydes	Acetaldehyde	0.015
	Hexanal[a]	0.005
	2-Hexenal[a]	0.017
Esters	Ethyl acetate	5.0
	Propyl propionate	0.057
	Butyl acetate	0.066
	2-Methylbutyl acetate	0.005
	Propyl butyrate	0.018
	Butyl propionate	0.025
	Ethyl butyrate	0.001
	Ethyl 2-methyl butyrate[a]	0.0001
	Ethyl pentanoate	0.005
	Pentyl acetate	0.005
	Hexyl acetate	0.002

[a] Compounds having apple or apple-like aromas according to panel.

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