Food Chemistry **19** (1986) 137–147

Charm Analysis of Apple Volatiles

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(Received: 13 June, 1985)

ABSTRACT

The 'Charm' analytical procedure was applied to several apple cultivars and replication gave almost identical charm responses. However, no two cultivars showed close resemblance by the technique. Charm response can be used, together with physical parameters, to describe apple flavour.

INTRODUCTION

Since the early studies of Powers & Chestnut (1920, 1922), many volatile compounds isolated from apples (*Malus domestica* Borkh) have been characterised (Schreier *et al.*, 1978; Williams *et al.*, 1980). However, knowledge of the biological activity of these chemical components is not abundant. Duerr (1981) examined the sensory impact of thirty known volatiles found in apples. None of the thirty correlated with any of the sensory variables that his panel had developed (Duerr, 1979) for apple odour. In a study of insect attractants, biological response of the apple maggot (*Rhagoletis pomonella* Walsh) to different apple volatiles was measured. Certain apple components attracted the insect to a model fruit (Fein *et al.*, 1982). However, in a further experiment, synthetic attractants were not as attractive as the apple itself (Reissig *et al.*, 1982). In both these studies it was assumed that all activity derived from known compounds; no attempt was made to measure the contribution made by components of

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Food Chemistry 0308-8146/86/$03·50

unknown chemical composition. In this paper we report odour-activity for both known and unknown components of apple.

In previous work (Acree *et al.*, 1984) we developed a bioassay to produce a measure of biological activity called charm. It quantitatively associates odour-activity with each component in a sample. In this paper, the composition of apples is examined in terms of odour-activity, and this activity is quantitatively associated with identified and unidentified components. Forty cultivars of apples, including those of commercial importance, were used to examine odour-activity in non-polar extracts of apple juice. Our results provide a chromatographical summary of essential odour quality in apples.

EXPERIMENTAL

Preparation of apple extracts

Forty different apple cultivars chosen for the experiment were grown at the New York State Agricultural Experiment Station. Each was harvested near its optimum maturity (Way, 1982). Table 1 lists cultivars used and the date at which they were harvested. A juice was pressed from each cultivar separately. To inhibit enzymatic changes in the volatile composition of the juice during preparation, whole apples were sliced into eighths, dipped in methanol and pressed immediately through a methanol-rinsed cheesecloth. A hydraulic press, using a stainless steel basket able to hold 0·5 to 1·0 kg of apples, delivered approximately 500 ml of juice into a flask containing 50 ml of methanol. Volatiles were extracted from each juice using the non polar solvent, 1,1,2-trichloro-1,2,2-trifluoro-ethane, by stirring at 60 rpm for 30 min. The Freon 113 layer was separated, dried using anhydrous $MgSO_4$, and concentrated in a rotary evaporator at 35 °C and 0·5 Pa to a 20-fold concentrate. Extracts were stored in amber glass bottles at 0 °C.

Bioassay: charm analysis

The charm bioassay combines sniffing of the gas chromatographic effluent with the measurement of paraffin retention indices. The technique measures odour intensity of extracted components in units of charm over a range of retention indices. Charm is the ratio of the amount of an odour-active compound to its detection threshold in a gas chromatographic

TABLE 1

Apple Cultivars used in the Experiment, their Harvest Data and Retention Indices of their Five Most Intense (Highest Charm) Odour Responses

Cultivar	*Harvest date (1982)*	*1st*	*2nd*	*3rd*	*4th*	*5th*
Paulared	13 Sep.	**1364**	1160	1624	844	**797**
Gravenstein	13 Sep.	**1366**	**964**	1161	1142	**1374**
Wealthy	13 Sep.	**1365**	1627	**961**	**788**	**1374**
Niagara	13 Sep.	**1365**	1627	**787**	**963**	**1374**
Jonamac	20 Sep.	1365	**962**	1627	843	**786**
Barry Crab Apple	21 Sep.	1629	**1365**	**788**	**1184**	963
Boller McIntosh	23 Sep. and 6 Oct.	**1368**	**788**	**964**	**1105**	844
Summerland McIntosh	23 Sep. and 6 Oct.	**1373**	**1359**	848	**792**	876
Macoun	6 Oct.	1357	**1370**	**1164**	**965**	**1224**
Cortland	6 Oct.	1634	845	**967**	**1373**	**1165**
Spartan	6 Oct.	**1370**	**1164**	1635	**966**	**785**
Otterson No. 1	6 Oct.	**1371**	**796**	875	**967**	**1166**
Liberty	8 Oct.	1357	**1368**	**1373**	**1190**	**787**
Empire	12 Oct.	**1367**	**1376**	1359	**968**	**787**
R. I. Greening	12 Oct.	**1374**	**969**	1384	**788**	1446
Holly	12 Oct.	**969**	**1374**	**1168**	**1362**	**790**
Jonathan	12 Oct.	1380	**970**	**790**	**1171**	1357
Cox Orange	12 Oct.	**1378**	**970**	849	**787**	**1170**
Jonagold	12 Oct.	**1378**	**1365**	1010	1387	**970**
Twenty Ounce	12 and 13 Oct.	**789**	**1377**	1196	**876**	**920**
Red Delicious	12 and 13 Oct.	**1379**	**1170**	**788**	971	1448
Jonalicious	12 and 13 Oct.	**1378**	**970**	**1171**	**1374**	1384
Jonared	12 and 13 Oct.	**1377**	1445	1231	**1362**	**969**
Monroe	12 and 13 Oct.	**1170**	972	**1376**	1384	**1365**
Melrose	20 Oct.	**788**	**878**	**1378**	**1363**	971
Spigold	20 Oct.	**969**	**1170**	**1362**	**1373**	1231
Golden Delicious	20 Oct.	**1362**	**1170**	1357	**969**	**1378**
Idared	20 Oct.	**1363**	**970**	**788**	**1169**	**1378**
Baldwin	25 Oct.	**1380**	**970**	**1364**	**789**	**1169**
York Imperial	25 Oct.	**877**	**962**	1384	1477	1488
Rome Beauty	25 Oct.	**968**	**1168**	**1363**	**1377**	972
Law Rome	25 Oct.	1381	972	**1171**	**1360**	**1365**
Stayman	25 Oct.	973	**1172**	**1366**	**790**	**880**
Winesap	25 Oct.	1380	**1172**	**1365**	971	974
Mutsu	25 Oct. and 2 Nov.	1380	972	**1367**	**879**	**1174**
Northern Spy	25 Oct. and 5 Nov.	973	1520	**1172**	**965**	**790**
Joan	2 Nov.	972	**1174**	**876**	**780**	1245
Ben Davis	2 Nov.	1380	972	**880**	1387	**1174**
Granny Smith	2 and 5 Nov.	**1172**	1379	**1365**	**971**	1234
Yellow Newtown	2 and 5 Nov.	973	1383	**1173**	**1366**	**790**

Bold numbers indicate retention indices for which an identity has been established.

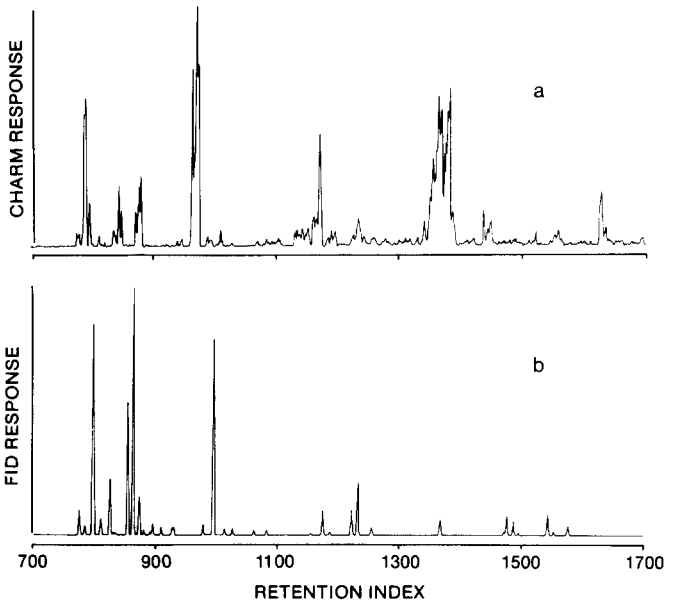

Fig. 1. A comparison between charm and flame ionisation response to extracts of forty cultivars of apples. Figure 1(a) is the average charm response chromatogram produced from separate analyses of the forty apple cultivars. Figure 1(b) shows the flame ionisation response (FID) to a combined sample of the same extracts. The chromatographic column was a 25 m × 0·35 mm fused silica coated with crossed-linked methyl silicone (0V101) and normal hydrocarbons were used for retention index standards.

effluent. A detailed description of charm is available in Acree *et al.*, (1984). For these experiments, a Hewlett Packard 5840 gas chromatograph was modified such that the effluent was mixed with a stream of humidified air (Acree *et al.*, 1976) and directed towards a subject equipped with a video terminal for recording responses. Duration of odour response was recorded, not perceived intensity. Intensity was computed from responses collected over repeated runs comprising three-fold serial dilutions. The dilutions were made from a 60-fold concentrate of the Freon extracts, and chromatographed on a 25 m by 0·35 mm fused silica column coated with cross-linked methyl silicone. Data were collected between 700 and 1800 *n*-paraffin retention indices. The resulting table of coincident responses contained the number of times, $n(i)$, odour was detected at the ith retention index. Charm was computed from this data as:

$$c(i) = 3^{n(i)-1}$$

and plotted against retention index. The resulting graphs, called charm

response chromatograms, relate the amount of a component in an extract, divided by its detection threshold in the gc effluent, to retention index. Figure 1(a) shows the average charm response chromatogram produced from the separate analyses of forty apple cultivars. Quantitative charm data were produced by integrating the charm function over limits defined by peaks in this chromatogram.

Chemical characterisation

A mixture was made from 1 ml of each extract, concentrated 100-fold, and used for chemical analysis. Unit mass spectra were obtained on a Hewlett Packard 5985 gas chromatograph–mass spectrometer and used to characterise the most odour-active constituents detected by charm analysis. Retention indices of authentic standards were determined in the same chromatograph under the same conditions as for the charm analysis (Acree *et al.*, 1984). Unless identification was based on the comparison of both retention indices and mass spectra with those of authentic standards, they were designated as tentative.

RESULTS AND DISCUSSION

Listed in Table 1 are the retention indices (RI) of the five most intense odours detected in the charm response chromatograms of each cultivar. The values in bold type are to within four RI of compounds identified in the combined extracts. The name and RI of these compounds are listed in Tables 2 and 3. Although many cultivars show odour-activity at a common RI, they differ at one or more RI and no single odour-activity was found common to all. Therefore, the odour of these apple cultivars cannot be described by simple variation in the concentration of a few chemicals. Furthermore, the data in Table 4, showing the top eleven charm responses for one cultivar compared with a replicate prepared and analysed at a different time, shows high precision. Only one of the top eleven responses was missing from the replicate, retention indices varied $\pm$ 0·9 units, and charm response varied $\pm$ 4·4%.

An average picture of apple odour is obtained by comparing sensory data with chemical data. Figure 1(a) shows the charm response chromatogram generated by averaging the data from apple cultivars listed in Table 1. It combines charm responses from extracts of the forty

TABLE 2

The Most Intense Odour-Activities (Charm) Detected in the Charm Response Chromatograms from Forty Apple Cultivars, Retention Indices of Standards Determined under Identical Chromatographical Conditions, and the Name of the Compounds Detected at the Standard Indices by Mass Spectroscopy

No.	*Charm*[a]		*Standards*	*Compounds detected*	*Mass spectroscopic match*
	%	*RI* ± 4	*RI* ± 1	*Name*	
1	11·8	1366	1366	β-damascenone	+
2	10·1	971		*75(100), 115(35), 83(20), 71(6)	−
3	9·4	1375	1369/1371	Hexyl hexanoate and butyl octanoate	+/+
4	7·5	788	784	Ethyl butyrate	+
5	6·7	1171	1175/1176	Butyl hexanoate and hexyl butyrate	+/+
				*Benzyl acetone and *benzothiazol	−/−
6	5·4	1353		*No spectra	−
7	4·4	965	965	6-Methylhept-5-en-2-one	+
8	3·8	1628		*No spectra	−
9	2·7	1161		*No spectra	−
10	2·5	880	881/883	Propyl butyrate and ethyl valerate	+/+
11	2·2	1386		*No spectra	
	No charm		1378	Ethyl decanoate	+
12	2·0	1232		*Isoamyl hexanoate	−
	68·5				

[a] Ordinates of the forty chromatograms were averaged at each retention index to produce the 'combined' chromatogram ('*' indicates unidentified compounds or tentative assignments).

cultivars of apples analysed separately at four dilutions: 3,000 sensory events experienced by a single person sniffing the effluent of 160 gas chromatographic runs is represented. Differences in the response of individual people to apple odour was not the object of the work described here.

For comparison, Fig. 1(b) shows the flame ionisation response (FID) chromatogram for a sample prepared by combining equal aliquots from the extracts of each cultivar. The combined charm response chromatogram shows an odour-active region located at retention index 970, but no corresponding peak of similar intensity is visible in the FID response chromatogram of the combined extract (Fig. 1(b)). This implies that the compound(s) responsible for odour in this region have low thresholds. The inverse is seen at retention index 1000, where a large FID response is

TABLE 3
Additional Odour-Activities and Their Associated Compounds

No.	*Charm*[a] %	*Charm*[a] *RI* ± 4	*Standards* *RI* ± 1	*Compounds detected* *Name*	*Mass spectroscopic Match*
1	1·4	796	795/800	Propyl propanoate and butyl acetate	+/+
2	1·0	961	956/957	1-Octene-3-one and *Isopentyl propanoate	+/−
3	0·90	835	834	Ethyl 2-methylbutyrate	+
4	0·67	1223	1222	Hexyl 2-methylbutyrate	+
5	0·59	1184	1182	Ethyl octanoate and *Decanal	+/+
6	0·47	1130	1123	Pentyl 2-methylbutyrate	+
7	0·46	988	985/982	Ethyl hexanoate and butyl butyrate and *isobutyl isopentanoate	+/+ −
8	0·43	1328	1322	Heptyl 2-methylbutyrate	+
9	0·42	1309	1307	Methyl decanoate	+
10	0·15	821	826	E-2-hexenal	+
11	0·15	1027	1027	Butyl 2-methylbutyrate	+
12	0·13	1208	1207	Methyl nonanoate	+
13	0·10	1079	1077	Pentyl butyrate and *Nonanal	+
14	0·10	861	862	3-Methylbutyl acetate	+
15	0·06	898	893/897	Butyl propanoate and pentyl acetate	+/+
16	0·06	1042		*3-Methylbutyl butyrate	−
17	0·02	763	761	Methyl 2-methylbutyrate and *isopropyl propanoate and *isobutyl acetate	+
18	0·01	710	706	Methyl butyrate	+
	5·81				

[a] Ordinates of the forty chromatograms were averaged at each retention index to produce the 'combined' chromatogram ('*' indicates unidentified compounds or tentative assignments).

seen but no charm is registered. The charm data draw attention away from sensorially inert components and focus analysis on the odour-activity.

Table 2 lists the twelve most odour-active peaks present in Fig. 1(a). Each contributed more than 2% charm and, when combined, all twelve contributed 69% of the total charm. Eight of these peaks were associated with compounds that could be responsible for their odour-activity. The assignments were made or verified by gas chromatographic and mass spectrometric analysis of the combined sample. The four remaining peaks were unidentified. The sixty-two response peaks in Fig. 1(a) with values greater than 0·01% accounted for 88% of the total charm.

TABLE 4
Replicated Runs of Charm Analysis of the Cultivar 'Ben Davis'

Retention indices			*Per cent difference*		
Rep. 1	*Rep. 2*	*Difference*	*Rep. 1*	*Rep. 2*	*Difference*
790	790	0	2·0	4·0	2·0
880	880	0	16·7	15·8	−0·9
972	972	0	5·1	20·0	14·9
974	974	0	13·6	5·1	−8·5
1171	1172	1	2·5	5·7	3·2
1233	1235	2	3·0	2·3	−0·7
1313	—	—	3·0	—	—
1379	1380	1	8·1	18·6	10·5
1386	1387	1	7·1	6·8	−0·3
1758	1757	−1	3·0	2·3	−0·7
1784	1787	3	4·0	0·6	−3·4

At RI 1366 the most intense odour (6·5 % charm) was detected. It was caused by beta-damascenone, as indicated by chemical and charm analysis of an authentic standard. Beta-damascenone has been found in many natural products, including apples (Nursten & Woolfe, 1972), and is thought to be a necessary, but non-characteristic, odour in most of them. It has a sweet, perfumy and fruity odour with a very low detection threshold in water (about two parts per 10^{12}, Braell, 1984). Masuda & Nishimura (1980), Williams *et al.* (1984) and Braell (1984) demonstrated that beta-damascenone is generally formed from an odourless precursor under post-harvest treatments. This study reveals that beta-damascenone also contributes odour to many apple cultivars prior to processing.

Two compounds occurring at RI 1171 and 790 were identified as hexyl butanoate and ethyl butanoate, respectively. Hexyl butanoate contributed 5 % charm and ethyl butanoate 3·5 %, and both have a fruity apple odour. At RI 1373, hexyl hexanoate, with an apple peel-like odour, contributed 5 % charm.

The twelve largest peaks also included five unidentified compounds with retention indices of 971, 1161, 1353, 1628 and 1386. One of these, at 971, had the second largest charm response and an herbaceous apple-like odour. Only a faint mass spectrum was visible at this RI in the combined extract. The other three unidentified peaks had odours described as miscellaneous, and possessed no visible spectra in the combined extract. Although ethyl decanoate was identified within seven RI of a 2·2 % charm

response at 1386, a separate charm analysis of standard ethyl decanoate produced no response even at ten times the concentration found in the extract. Therefore, some other compound with a much lower threshold must be the cause of this charm.

Table 3 lists an additional twenty-seven compounds identified in the combined apple extract that could be associated with one of the responses shown in Fig. 1. However, the total charm that can be explained by these compounds is less than 6%. Ethyl-2-methyl butanoate, RI 835, was reported as the characteristic odour component of the cultivar 'Golden Delicious' (Flath *et al.*, 1967). However, ethyl-2-methyl butanoate contributed less than 1% charm in the combined charm response chromatogram, and contributed only 1·1% to the individual chromatogram of 'Golden Delicious'. Although this compound seems to be a minor contributor to odour at harvest, it may become more important as a result of post-harvest changes in apple volatile composition.

Finally, of the 6-carbon aldehydes and alcohols reported in apples (Koch *et al.*, 1976; Jepson, 1978; Panasiuk *et al.*, 1980; Pyysalo *et al.*, 1980; Duerr, 1981), only hexanol and 3-(*Z*)-hexenol were detected in the combined extract, with only 0·6% and 0·7% charm, respectively. These compounds are lipid oxidation products formed when apples are damaged or crushed. However, the methanol used in this study inhibited these processes. The results obtained here provide a picture of the odour contained in intact fruit at maturity. For certain apple products, these compounds would be expected to make a much greater contribution to odour.

CONCLUSIONS

This study shows that odour in apples is not caused by the same compounds in all cultivars. Although replication of one cultivar gave almost identical charm response chromatograms, no two cultivars showed close resemblance. A generalised description of apple odour produced by combining samples showed beta-damascenone, butyl, isoamyl and hexyl hexanoates, along with ethyl, propyl and hexyl butanoates, to be important to the odour of most cultivars. Although these compounds have been identified previously in apples, five of the twelve odours detected in this experiment could not be associated with any known apple component.

The additional twenty-seven compounds identified in the composite sample were chemicals identified with apple flavour. Some of these did contribute significantly to odour in certain cultivars; however, they could only be associated with weaker, or less frequently occurring, odour responses. The remaining thirty-six odour responses could not be associated with any known structure.

The results emerging from charm analysis are two dimensional. The first dimension consists of physical parameters which are particularly useful for the chemical characterisation of unknown components. Charm response, the second dimension, is a quantitative measure of biological response to odour stimuli. Together, these two dimensions provide a concise description of apple flavour.

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