

リンゴ果実の香気成分の検索と品種特性

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Composition of Volatile Compounds of Apple Fruits in Relation to Cultivars¹

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Summary

Experiments were carried out to analyse the cultivar differences in the composition and content of flavour compounds by applying the head space and high vacuum distillation methods.

1. Seventy to 80 compounds were detected in the gas chromatogram peaks of which 39 compounds were identified by gas chromatography-mass spectrometry.

2. The classes of the volatile compounds identified included 27 kinds of esters, 6 of alcohols, 2 of aldehydes and hydrocarbons, a phenol and an acid.

3. Esters were the most abundant flavour components detected when the head space method was applied, accounting for 80% or more of the total content of volatile compounds. And the total amounts of volatile compounds were highest in 'Hatsuaki' (1.889 ppm) followed in descending order by, 'Kogyoku' (1.531 ppm), 'Golden Delicious' (0.196 ppm), 'Mutsu' (0.187 ppm) and 'Fuji' (0.055 ppm).

4. The amount and composition of volatile compounds estimated by the high vacuum distillation method were nearly the same as those by the head space method. However, in the former method the class of alcohols was quantitatively the most abundant, the percentage of total volatile compounds ranging from 53.3% in 'Hatsuaki' to 75.5% in 'Fuji'.

5. The total amounts of volatile compounds recovered by the high vacuum distillation method were highest in the cultivar 'Kogyoku', 9.415 ppm, followed by 'Hatsuaki' with 8.936 ppm, 'Golden Delicious' with 5.964 ppm, 'Mutsu' with 3.711 ppm and lastly 'Fuji' with 2.273 ppm.

6. From the above results it can be concluded that the cultivar 'Kogyoku' and related cultivar 'Hatsuaki' contain higher levels of volatile compounds and show a higher aroma intensity than other cultivars.

Introduction

Numerous investigations have been carried about the composition of the volatile compounds of apples, since the flavour of apples

is a very important quality determination factor(1~7, 9~11, 17). Power and Chesnut (13, 14) reported for the first time that the aroma constituents of several varieties of apples consisted mainly of pentyl esters of formic, acetic and hexanoic acids, a considerable portion of acetaldehyde, geraniol and

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traces of methyl and ethyl alcohol.

Flath *et al.* (2) studied the volatile compounds of 'Delicious' apples, and pointed out that ethyl 2-methylbutyrate, hexanal and 2-hexanal were essential to the apple aroma. Katayama *et al.* (7) observed the presence of 15 compounds of esters and alcohols, and also Yajima *et al.* (18) identified sixty-seven compounds of hydrocarbons, alcohols, aldehydes, ketones, esters and miscellaneous compounds from the 'Kogyoku' ('Jonathan') apple juice and peels.

However the content and composition of the volatile compounds obtained from apple fruit differ markedly from study to study depending on the methods of determination and apple cultivars employed. To our knowledge, reports on differences of flavour in cultivars are not available except for the report stating that 'Kogyoku' shows a high intensity of aroma and is suitable for juice making (7).

The present study was undertaken to analyse the differences in the composition and content of the flavour compounds of five main cultivars in Japan and to obtain the suitability of the cultivars for processing along with establishing an index for breeding programs relating to flavour.

Materials and Methods

Materials

Apples of five cultivars 'Hatsuaki' (picking date Sept. 26 th), 'Kogyoku' (Oct. 17 th), 'Golden Delicious' (Oct. 27 th), 'Mutsu' (Oct. 31 st), 'Fuji' (Nov. 5 th) were harvested in the orchard of the Morioka Branch of the National Fruit Tree Research Station in Mo-

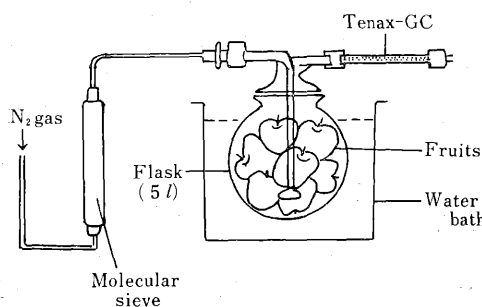


Fig. 1. Apparatus for collecting volatile compounds from apple fruits by applying the head space method.

rioka Prefecture in 1983 and examined within 10 days after picking when considered ready for marketing.

Determination of volatile compounds

Volatile compounds were isolated by a modification of the head space method (5) and high vacuum distillation method (7) developed previously by Jennings and Katayama *et al.* respectively.

Head space method: Seven to 9 fruits (1.72~2.8 kg) were placed in the apparatus illustrated in Figure 1 and kept at 20°C by using a water bath controlled by a thermostat. Nitrogen gas (50~60 ml/min) which passed through a molecular sieve was blown over the apples for 24 hours. The volatile compounds in the emerging gas stream were absorbed by two grams of Tenax-GC.

The volatile compounds trapped on the Tenax-GC were dissolved in ethyl ether. After the evaporation of ethyl ether by bubbling in purified nitrogen gas, the residual volatile compounds were dissolved in 10 μ l of *n*-hexane containing 10% butyl benzene as an internal standard, and part of this solution was injected into a gas chromatograph.

High vacuum distillation method: A modification of the method developed previously by Katayama *et al.* (7) was applied in this experiment. Seven or 9 apples were used for analysis. Each fruit was cut into quarters and cored to give 2 kg or more of fruit flesh. Two kg of the flesh were homogenized with 400 ml water containing 20 g of NaCl under cooled conditions to inhibit enzymatic activity (5, 15).

All of the homogenate was poured into a 5 l flask illustrated in Figure 2. The flask was connected to a set of four cold traps at the end of which stood a vacuum pump. The volatile compounds were distilled off under the reduced pressure of 0.1~0.2 mm Hg by bubbling the purified nitrogen gas at the temperature of 35°C for 6 hours.

Evaporated volatile compounds under reduced pressure were caught by sequential trapping in cooled ethyl alcohol, dry ice and dry ice-acetone. Cold trap condensate con-

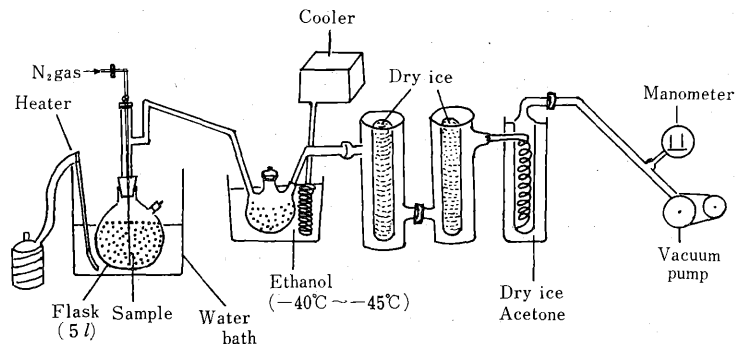


Fig. 2. Apparatus for collecting volatile from apple juice by applying the high vacuum distillation method.

taining water was collected together, the apparatus was rinsed with 100 ml of ethyl ether and the two solutions were mixed. The mixed solution was salted out by 200 g of NaCl, and the water phase washed three times with ethyl ether (200 ml). This solution was added to the original ether solution to obtain 300 ml of extracting solution for the volatile compounds.

The ethyl ether solution was dehydrated by 30 g of magnesium sulfate. After filtration of this solution, ethyl ether was evaporated by a rotary evaporator at a low temperature to obtain the volatile compounds as a residue. The residue was dissolved into 300 μ l of *n*-hexane containing butyl benzene as internal standard and analysed by gas-chromatography.

Butyl benzene was selected as the internal standard since its retention time coincided with an uncrowded region of the apple flavour chromatogram and since it was not a constituent of apple juice.

Gas chromatography and gas chromatography-mass spectrometry

Gas chromatographic data were obtained using a Shimadzu GC-9 A gas chromatograph fitted with a flame ionization detector and using 50 m \times 0.25 mm fused silica capillary columns coated with FFAP (WCOT, Gaschromat. Ind.). The instrument was operated at a nitrogen flow of 0.8 ml/min and the column oven temperature of 70° to 200°C was programmed at a rate of 3°C/min with a sample size of 0.1 μ l.

A Hewlett Packard 5992 B GC-MS system

scanning at 70 ev was used to record mass spectra data. The columns were similar to those used for analytical gas chromatography. After an initial isothermal period of 3 min the columns were programmed from 100° to 200°C at a rate of increase of 5°C/min. Volatile compounds were indicated in ppm of the fresh weight.

Authentic samples were obtained for all the compounds detected in the apple volatile substances and identified by retention time and mass spectral analysis. Retention times of these compounds were determined under the gas chromatographic condition described above to identify the volatile compounds of apple fruit samples.

Results and Discussion

Volatile compounds identified by the application of the head space method

A typical gas chromatographic pattern of the volatile compounds of 'Kogyoku' fruits obtained by the head space method is illustrated in Figure 3. Seventy to 80 compounds were detected in the gas chromatogram peaks of which 39 compounds were identified by gas chromatography-mass spectrometry. The classes of compounds identified included 27 kinds of esters, 6 of alcohols, 2 of aldehydes, 2 of hydrocarbons, a phenol and an acid.

In these classes of compounds, the most abundant volatile constituents consisted of esters. Alcohols, aldehydes, hydrocarbons, phenol and acid were minor compounds in quantity. Of the volatile compounds detect-

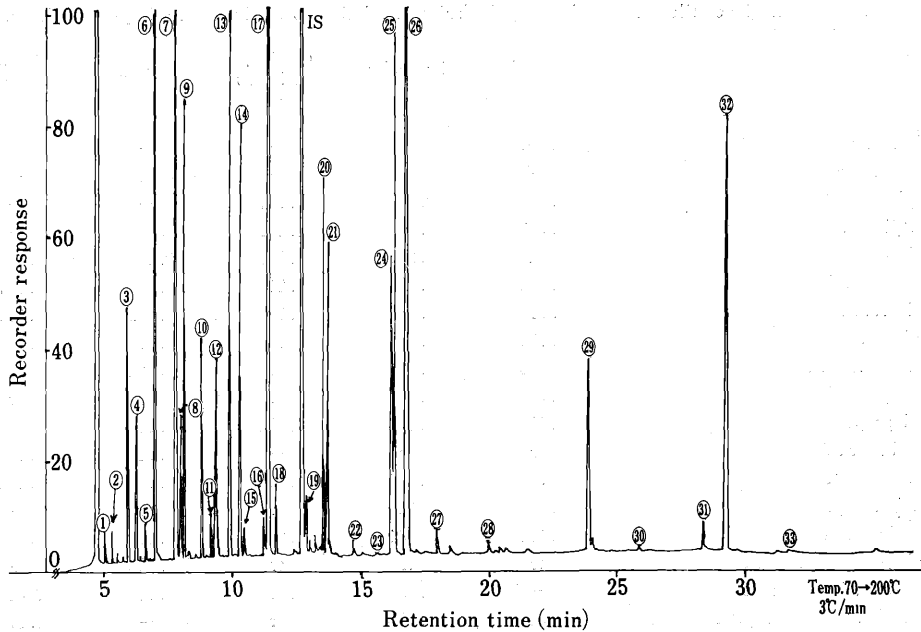


Fig. 3. Chromatogram of head space volatile concentrate from 'Kogyoku' apple.

Peak	Compound
1.	Methyl acetate
2.	Ethyl acetate
3.	Propyl acetate
4.	Isopropyl acetate
5.	Ethyl 2-methyl butyrate
6.	Butyl acetate
7.	2-Methyl butyl acetate
8.	Butyl alcohol
9.	Butyl propionate
10.	Amyl acetate
11.	2-Met. but. propionate
12.	2-Met. but. alcohol
13.	Butyl butyrate
14.	Butyl 2-methyl butyrate
15.	Amyl alcohol
16.	2-Met. but. butyrate
17.	Hexyl acetate
18.	2-Methyl butyl 2-met. But.
19.	Propyl caproate
20.	Hexyl propionate
21.	Hexyl alcohol
22.	Isobutyl caproate
23.	trans-2-hexenyl alcohol
24.	Butyl caproate
25.	Hexyl butyrate
26.	Hexyl 2-methyl butyrate
27.	2-Methyl butyl caproate
28.	Amyl caproate
29.	Hexyl caproate
30.	Methyl chavicol
31.	trans, cis- α -Farnesene
32.	trans, trans- α -Farnesene
33.	Hexyl caprylate

IS=Internal standard (Butyl benzene)

ed and identified in the cultivars used, butyl acetate, 2-methylbutyl acetate, hexyl acetate, hexyl propionate, hexyl butyrate and hexyl 2-methylbutyrate were found to be the major constituents accounting for the quantity.

Compared with the results previously obtained(1~3, 7, 12, 15), the present data were in agreement in the case of the esters of methyl acetate, ethyl acetate, propyl acetate, butyl acetate, hexyl acetate, butyl propionate, hexyl propionate, ethyl butyrate, butyl butyrate, ethyl 2-methylbutyrate and also the alcohols comprising isobutyl alcohol, butyl alcohol, 2-methylbutyl alcohol, hexyl alcohol, trans-2-hexenyl alcohol. But, 4-methoxyallylbenzene which contributes to the spice-

like characteristic of the aroma(17) was not detected in this experiment.

Some characteristic features of the individual cultivars in relation to the composition and content of the volatile compounds are shown in Table 1. Higher concentration of methyl chavicol of the phenolic compound was a characteristic of the cultivar 'Hatsuaki'. Cultivars 'Hatsuaki' and 'Kogyoku' contained larger quantities of esters; with comparatively higher amounts of alcohols.

The cultivar 'Mutsu' contained esters, namely ethyl butyrate and ethyl 2-methylbutyrate. While many of the compounds may contribute to the apple-like aroma, it can be considered that ethyl 2-methylbutyrate is responsible for the special aroma of

Table 1. Content of volatile compounds of apple fruits in relation to cultivars (head space method).

	Hatsuaki	Kogyoku	Golden. Delicious	Mutsu	Fuji
Esters					
Methyl acetate	0.001	+	+	+	+
Ethyl acetate	+	+	+	0.005	+
Propyl acetate	0.002	0.012	0.001	+	+
Isobutyl acetate	0.003	0.008	0.002	0.001	+
Butyl acetate	0.140	0.242	0.086	0.034	0.005
2-Methylbutyl acetate	0.182	0.300	0.016	0.008	0.011
Amyl acetate	0.015	0.013	0.003	0.002	0.001
Hexyl acetate	0.532	0.443	0.036	0.028	0.003
Butyl propionate	0.040	0.027	0.004	0.002	0.002
2-Methylbutyl propionate	0.005	0.003	tr	tr	tr
Hexyl propionate	0.183	0.028	0.002	0.002	0.001
Ethyl butyrate	tr	tr	+	0.018	0.001
Butyl butyrate	0.033	0.037	0.007	0.005	0.001
2-Methylbutyl butyrate	0.002	0.003	tr	tr	+
Hexyl butyrate	0.166	0.041	0.006	0.008	0.001
Ethyl 2-methylbutyrate	tr	tr	tr	0.003	+
Butyl 2-methylbutyrate	(0.031)	(0.029)	(0.004)	(0.005)	(0.002)
2-Methylbutyl 2-methylbutyrate	0.003	0.005	tr	+	0.001
Hexyl 2-methylbutyrate	0.254	0.157	0.006	0.014	0.007
Ethyl caproate	(0.031)	(0.029)	(0.004)	(0.005)	(0.002)
Propyl caproate	+	+	tr	+	tr
Isobutyl caproate	+	+	tr	tr	tr
Butyl caproate	0.031	0.027	0.009	0.011	0.003
2-Methylbutyl caproate	0.001	0.002	tr	+	0.001
Amyl caproate	0.006	0.002	0.003	+	0.001
Hexyl caproate	0.052	0.018	tr	0.009	0.002
Hexyl caprylate	0.001	+	tr	+	+
Alcohols					
Isobutyl alcohol	+	+	tr	tr	tr
Butyl alcohol	0.003	0.011	0.002	0.002	0.001
2-Methylbutyl alcohol	0.004	0.017	+	+	0.001
Amyl alcohol	0.003	0.001	+	tr	+
Hexyl alcohol	0.017	0.030	+	0.001	0.001
trans-2-Hexenyl alcohol	0.001	+	+	+	+
Aldehydes					
Hexyl aldehyde	tr	tr	tr	tr	tr
trans-2-Hexenyl aldehyde	tr	tr	tr	tr	tr
Hydrocarbons					
trans, cis- α -Farnesene	0.008	0.003	+	0.002	0.001
trans, trans- α -Farnesene	0.114	0.043	0.005	0.022	0.006
Phenol					
Methyl chavicol	0.025	+	+	+	+
Acid					
2-Methylbutyric acid	tr	tr	tr	tr	tr
Total (ppm)	1.889	1.531	0.196	0.187	0.055

+ : Below 0.001 ppm () : Double peaks which shared equal amount of volatiles

tr : trace

apples as shown by Flath *et al.* (2) in 'Delicious' apples.

The parents of the cultivar 'Hatsuaki' are 'Kogyoku' and 'Golden Delicious' apples. The production of volatile compounds by 'Hatsuaki' more nearly resembles that of 'Kogyoku' as shown in Figure 4, with the exception of the content of methyl chavicol. Also these two cultivars gave a similar or-

ganoleptic evaluation with a high aroma intensity. The volatile compounds of the cultivar 'Mutsu' also resemble those of their parental cultivar, 'Golden Delicious'. Study of the volatile compounds in a known percentage may provide criteria for evaluating breeding lines and programs for the improvement of flavour in cultivars.

Cultivar differences in the percentage of component part of volatile compounds estimated by the head space method are also illustrated in Figure 5. The high percentage of esters recovered by this method which ranged from 81.8% ('Fuji') to 96.4% ('Golden Delicious') (as indicated in Figure 5) may indicate the ability of esterification in the fruits themselves, since the volatile compounds emanated from fresh whole fruit.

As mentioned above, esters are the most abundant volatile compounds especially in the cultivars 'Hatsuaki' and 'Kogyoku'. Schreier *et al.* (16) classified the apple cultivars with volatile compounds into the ester type and the alcohol type of apples. Results obtained in the cultivar 'Kogyoku' ('Janathan') and related cultivar 'Hatsuaki' agreed with the report of Schreier who stated these cultivars belong to the ester type.

As shown in Table 1 and Figure 4, the total content of volatile compounds was the highest in 'Hatsuaki' (1.889 ppm), followed in descending order by 'Kogyoku' (1.531 ppm), 'Golden Delicious' (0.196 ppm), 'Mutsu' (0.187 ppm) and 'Fuji' (0.055 ppm). The cultivar 'Hatsuaki' contained 35 times the amount of volatile compounds of 'Fuji' which was the lowest among the cultivars examined.

Brown *et al.* (1) reported that many volatile substances reached maximum levels at a time which nearly coincides with the respiratory climacteric. Although the respiration climacteric was not estimated in the present materials, it can be concluded that individual amounts of volatile substances reflect the cultivar differences as the fruits were used 10 days after the optimum picking time for every cultivars.

Cultivar differences of volatile compounds shown in Figure 4, suggested that the

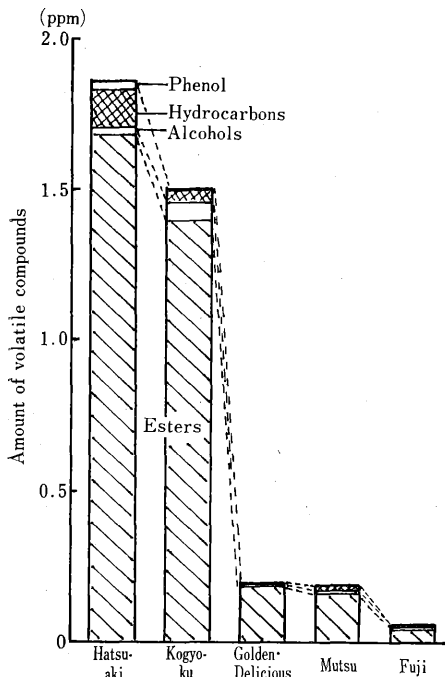


Fig. 4. Cultivar difference in each class of volatile compounds estimated by the head space method.

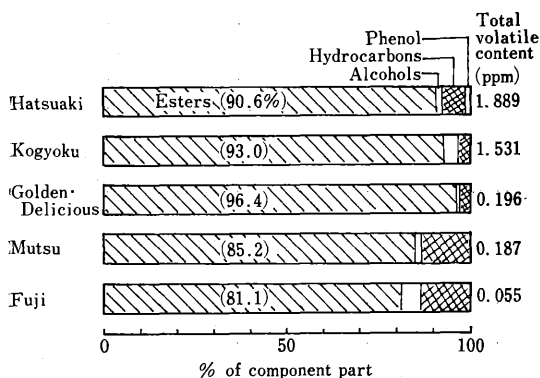


Fig. 5. Percentage of component part of each class of volatile compounds estimated by the head space method.

Table 2. Content of volatile compounds of apple fruit juice in relation to cultivars (high vacuum distillation method).

	Hatsuaki	Kogyoku	Golden. Delicious	Mutsu	Fuji
Esters					
Methyl acetate	+	+	+	+	+
Ethyl acetate	0.027	0.022	0.011	0.011	0.010
Propyl acetate	+	+	+	tr	tr
Isobutyl acetate	+	+	+	+	+
Butyl acetate	0.839	0.417	0.562	0.106	0.023
2-Methylbutyl acetate	0.450	0.472	0.190	0.048	0.135
Amyl acetate	0.042	0.016	0.027	+	+
Hexyl acetate	1.376	0.478	0.684	0.178	0.043
Butyl propionate	0.026	0.026	0.026	+	+
2-Methylbutyl propionate	tr	tr	tr	tr	tr
Hexyl propionate	0.023	+	0.009	tr	tr
Ethyl butyrate	tr	tr	tr	0.064	+
Butyl butyrate	tr	tr	tr	tr	tr
2-Methylbutyl butyrate	tr	tr	tr	tr	tr
Hexyl butyrate	0.057	0.021	0.067	0.034	tr
Ethyl 2-methylbutyrate	tr	tr	tr	0.014	+
Butyl 2-methylbutyrate	(+)	(+)	(0.030)	(0.043)	(0.001)
2-Methylbutyl 2-methylbutyrate	tr	tr	tr	tr	tr
Hexyl 2-methylbutyrate	0.074	0.053	0.049	0.053	0.029
Ethyl caproate	(+)	(+)	(0.030)	(0.043)	(0.001)
Propyl caproate	+	tr	tr	tr	tr
Isobutyl caproate	+	0.016	0.009	tr	tr
Butyl caproate	0.013	0.015	0.038	0.024	0.011
2-Methylbutyl caproate	tr	tr	tr	tr	tr
Amyl caproate	tr	tr	tr	tr	tr
Hexyl caproate	0.024	tr	0.019	0.026	tr
Hexyl caprylate	tr	tr	tr	tr	tr
Alcohols					
Isobutyl alcohol	0.067	0.135	0.017	0.022	0.011
Butyl alcohol	2.367	2.688	1.248	0.824	0.265
2-Methylbutyl alcohol	0.458	1.501	0.095	0.169	0.408
Amyl alcohol	0.032	0.031	0.017	0.022	0.013
Hexyl alcohol	1.653	1.804	1.323	1.220	0.598
trans-2-Hexenyl alcohol	0.188	0.403	0.166	0.298	0.426
Aldehydes					
Hexyl aldehyde	0.320	0.141	0.356	0.088	0.028
trans-2-Hexenyl aldehyde	0.846	0.894	0.913	0.388	0.258
Hydrocarbons					
trans, cis- α -Farnesene	tr	tr	tr	tr	tr
trans, trans- α -Farnesene	0.020	0.024	0.049	0.036	0.013
Phenol					
Methyl chavicol	tr	tr	tr	tr	tr
Acid					
2-Methylbutyric acid	0.034	0.258	0.029	+	+
Total (ppm)	8.936	9.415	5.964	3.711	2.273

+ : Below 0.001 ppm () : Double peaks which shared equal amount of volatiles

tr : trace

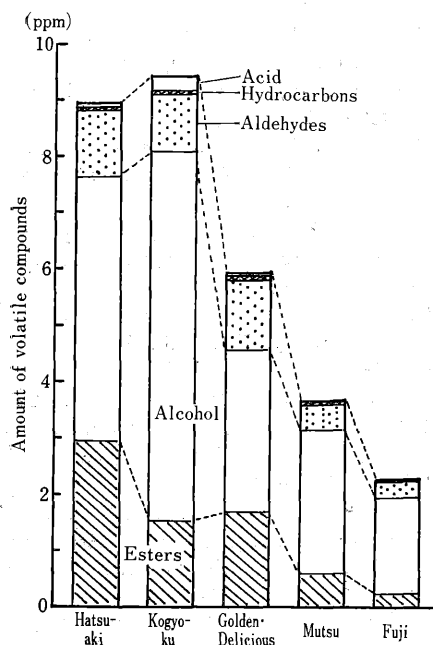


Fig. 6. Cultivar differences in each class of volatile compounds estimated by the high vacuum distillation method.

'Hatsuaki' and 'Kogyoku' have a high ability of emanation of volatile compounds from the whole fruit. In taking into account the organoleptic evaluation of apple flavour in relation to the method of recovery, the head space method may be more suitable for the estimation of flavour intensity by olfactory tests.

Volatile compounds identified by the application of the high vacuum distillation method

Table 2 shows the amounts of volatile compounds detected from the fruit homogenate of the respective cultivars by the high vacuum distillation and gas chromatographic method.

Few differences were observed in the composition and content of the volatile compounds detected by the high vacuum distillation method compared to the head space method. Esters of hexyl acetate, butyl acetate, 2-methylbutyl acetate and alcohols comprising butyl alcohol, hexyl alcohol were the major components estimated by the high vacuum distillation method. In contrast, the total amount of volatile compounds recovered by the high vacuum distillation method for

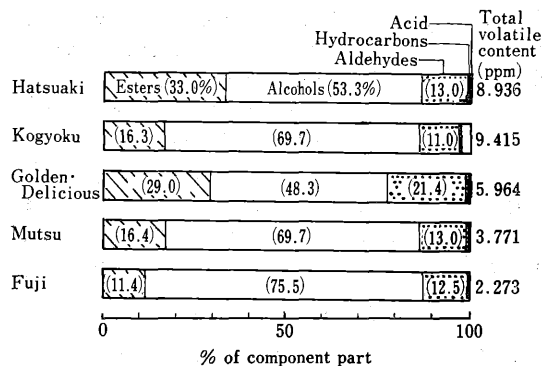


Fig. 7. Percentage of component part of each class of volatile compounds estimated by the high vacuum distillation method.

6 hours was higher than that of the volatile compounds collected for 24 hours by the head space method.

Figures 6 and 7 indicate the total contents of volatile compounds or percentage of individual classes of esters, alcohols, aldehydes, hydrocarbons, phenol and acid, respectively. It was recognized that the class of alcohols was the most abundant (Figure 6) showing a percentage ranging from 53.3% in 'Hatsuaki' to 75.5% in 'Fuji' (Figure 7). On the other hand, the percentage of esters which accounted for 80% or more of the total content in the case of the head space method was very low ranging from 11.4% in 'Fuji' to 33.3% in 'Hatsuaki' when the high vacuum distillation method was applied. In the other classes of volatile compounds, the concentrations were very low except for the aldehydes which showed a relatively high content compared to that detected by the head space method.

The reason why the content of the esters is low in the high vacuum distillation method may be ascribed to the fact that the esters did not evolve continuously, since the fruit was crushed and lost the ability of esterification as in the case of controlled atmosphere storage(8). The total amounts of volatile compounds recovered by the high vacuum distillation method were highest in the cultivar 'Kogyoku', 9.415 ppm (Table 2) followed by 'Hatsuaki' 8.936 ppm, 'Golden Delicious' 5.964 ppm, 'Mutsu' 3.711 ppm and lastly 'Fuji'

ji' 2. 273 ppm. This pattern of volatile content was found to be in agreement with the organoleptic tests of the cultivars used.

It has been shown that the cultivars 'Kogyoku' and 'Hatsuaki' both of which contain high levels of volatile compounds may be used for the improvement of the flavour in fruit processing and breeding programs.

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リンゴ果実の香気成分の検索と品種特性

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摘 要

リンゴの香気にかかわる品種の育成や利用加工上の指針を確立するため、主要リンゴ5品種の生鮮果実の香気成分の検索と品種特性について、丸ごと果実のヘッドスペース法と破碎果実(生鮮果汁)の高真空蒸留法により香気成分を回収して検討した。

1. ヘッドスペース法による香気回収結果から、70~80の成分がガスクロマトーンとして検出され、そのうち39成分をガスクロマトグラフ及びガスクロマトグラフ・質量分析計により同定した。

2. 同定された39成分の内訳は、エステル類27、アルコール類6、アルデヒド類2、炭化水素類2、フェノール類1及び酸類成分であった。

3. ヘッドスペース法による全香気成分のうち、エステル類は全品種とも80%以上を占めた。また、全香気成分含有量は‘はつあき’(1.889 ppm)>‘紅玉’(1.531)>

‘ゴールデン・デリシャス’(0.196)・‘陸奥’(0.187)>‘ふじ’(0.055)の順であった。

4. 高真空蒸留法に基づく香気成分組成もヘッドスペース法によるそれとほとんど同様であった。しかし、高真空蒸留法による香気成分組成はアルコール類が最も多く、‘はつあき’の53.3%から‘ふじ’の75.5%に及んだ。

5. 高真空蒸留法による全香気成分の回収量は、‘紅玉’(9.415 ppm)>‘はつあき’(8.936)>‘ゴールデン・デリシャス’(5.964)>‘陸奥’(3.711)>‘ふじ’(2.273)の順であった。

6. 以上の結果から、‘紅玉’やその血縁の‘はつあき’種の香気成分は、香気回収方法を問わず、他の3品種より著しく多く、香り立ちの良い原因であることが判明した。