The Aroma Constituents of "KOGYOKU" Apple

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Investigation was carried out about the aroma constituents of "KOGYOKU" apples. After ethereal extraction and fractional distillation, 23 g of neutral oil was obtained from 21.7 kg of commercial natural flavour essence. Carbonyl compounds remained mostly in aqueous phase. The yield was 0.0007% of the original fruits in weight basis.

The following compounds were isolated; ethyl alcohol, *n*-propyl alcohol, *n*-butyl alcohol, isobutyl alcohol, *n*-amyl alcohol, (–)-2-methyl butanol, *n*-hexyl alcohol, hexene-3-ol, *n*-propyl acetate, *n*-butyl acetate, *n*-amyl acetate, *n*-hexyl acetate, ethyl caproate, formaldehyde, acetaldehyde, *n*-propionaldehyde, *n*-butyraldehyde, 2-hexenal, nonyl aldehyde, isovaleric acid, caproic acid, benzoic acid, and an unknown α,β -unsaturated acid.

INTRODUCTION

Numbers of investigations have been made about aroma constituents of apples since 1920. Power and Chesnut¹⁾ found amyl esters of formic, acetic, caproic, caprylic acids and considerable portion of acetaldehyde, traces of methyl alcohol, ethyl alcohol, and formic, acetic, caproic acids in several varieties. Later²⁾, they reported the presence of geraniol in McIntosh apples.

The improvement of chromatographic technique and of recovering process of flavour constituents from fruit juice brought about the progress of these studies. White³⁾ identified acetaldehyde, acetone, hexyl aldehyde, furfural, 2-hexenal, methyl alcohol, ethyl alcohol, *n*-propyl alcohol, isopropyl alcohol, *n*-butyl alcohol, 2-methyl butanol, hexyl alcohol, and methyl, ethyl, isopropyl, *n*-butyl esters of formic, acetic, propionic, butyric acids from the mixture of McIntosh and Stayman Winsap apples. Thompson⁴⁾ found

methyl and ethyl esters of formic, acetic, valeric, caproic acids in Granny Smith apples.

Present work concerns with aroma constituents of commercial apple flavour essence^{*1)} which was obtained from "KOGYOKU" apples^{*2)} at Aomoriken Kaju Co., Ltd. by the use of recovery unit. The essence has a fine fresh apple aroma, and the concentration was 150 fold.

EXPERIMENTAL AND RESULTS

A total of 21.7 kg of apple flavour essence was saturated with ammonium sulfate and extracted three times with ether (total 4.5 l). Upon treatment of the aqueous layer with 2,4-dinitrophenylhydrazine, 8 g of the hydrazone was obtained. The ethereal extracts were combined and washed three times with sodium carbonate solution. A small amount of a mixture of volatile acids was recovered from sodium carbonate solution. The ethereal extract was concentrated by fractional

1) F.B. Power and V.K. Chesnut, *J. Am. Chem. Soc.*, **42**, 1509 (1920).

2) F.B. Power and V.K. Chesnut, *ibid.*, **44**, 2938 (1922).

3) J.W. White, Jr., *Food Research*, **15**, 68 (1950).

4) A.R. Thompson, *Aust. J. Sci. Res., B*, **4**, 283 (1951).

*1) According to the information from the factory, this specimen is quite free from any additive compounds.

*2) "KOGYOKU" is one of the most popular species in our country which corresponds to American Jonathan apples, transplanted to Japan in about 1872.

distillation and the residue was dissolved in a small amount of *n*-pentane. The pentane solution was extracted three times with water to remove water-soluble alcohols. After removal of pentane, 23 g of apple oil was obtained. The yield was 0.0007% of the original fruits. The procedures described above are summarized in Diagram 1.

Neutral oil was fractionally distilled by means of Podbielniak's 8 mm × 12 inch Heli-Grid column under reduced pressure. Ten fractions were collected and the residue was steam distilled to recover volatile substances. The results were summarized in Table I.

TABLE I. FRACTION OF THE NEUTRAL APPLE OIL

Fraction No.	Volume ml.	Calcd. b.p (760 mm)
Cold trap condensate	3.0	—
1	1.0	103~109
2	1.0	109~113
3	2.0	113~115
4	1.0	115~124
5	1.0	124~127
6	1.0	127
7	1.0	127~138
8	1.0	138~143
9	1.0	143~144
10	0.5	144~147
Steam-distd. fraction	1.8	—
Residue	4.5	—

Analyses were carried out by the use of Shimadzu's GC-2B type gas chromatograph. The column materials were polyethylene glycol-celite and silicon grease-celite.

The composition of each fraction was examined at first by referring to the IR spectrum and the retention time of the two sets of gas chromatography by the use of the two sorts of column material described above. The fractions which seemed to contain alcohols were saponified with the mixture of ethylene glycol, water and potassium hydroxide (6:1:1). By referring to gas chromatograms of both samples, before and after hydrolyses, final estimation of each ester was attained. Owing to the rather complicated compositions of fraction 8, 9, 10 and of the parts recovered from the distillation residue, column chromatographic separation was applied to these fractions by using Mallinckrodt's silicic acid as the adsorbent and *n*-hexane solution of ether (3%, 5%, 10%, 30%) as the elution solvent. Each fraction thus obtained was followed by the analyses mentioned above.

It was found that both cold trap condensate and fraction 1 consisted of isobutyl alcohol, *n*-butyl alcohol, *n*-propyl acetate, and *n*-butyl acetate. The gas chromatograms of trap condensate, before and after hydrolyses, were shown in Fig. 1. Column chromatography was not practical for these fractions because of their high volatility.

Fractions from 2 to 5 contained *n*-butyl alcohol, (–)-2-methyl butanol and *n*-butyl acetate.

Fractions 6 and 7 were (–)-2-methyl butanol in pure state. Fraction 8 was a mixture of (–)-2-methyl butanol, *n*-amyl alcohol, *n*-hexyl alcohol, hexene-3-ol, ethyl caproate, and hexyl acetate. From fractions 9, 10 and steam-distilled fraction, hexyl alcohol and a small amount of hexene-3-ol were isolated.

FIG. 1. The Gas Chromatogram of Trap Condensate.

- A. Before hydrolyses, B. After hydrolyses.
 1. isobutyl alcohol 4. *n*-butyl acetate
 2. *n*-butyl alcohol 5. *n*-propyl alcohol
 3. *n*-propyl acetate

Column material: Silicon grease-celite 2 m.
 Temp.: 130°C. Carriergas: N₂

The retention time in gas chromatography*³⁾ these compounds are: *n*-butyl alcohol, 5.4; (–)-2-methyl butanol, 6.0; *n*-butyl acetate, 4.9; *n*-amyl alcohol, 7.5; *n*-hexyl alcohol, 9.0; hexene-3-ol, 10.1; *n*-hexyl acetate, 7.5; ethyl caproate, 6.8.

The precipitates of 2,4-dinitrophenylhydrazones from aqueous layer after ethereal extraction of the essence were subjected to column chromatography on silicic acid. As the developing solvent, the mixture of ether and *n*-hexane was used in the ratio 1:9 at the beginning and then 3:7. Each adsorption zone developed on the column was scrubbed out separately with long spatula and eluted out with ether. Analyses were carried out by means of paper chromatography using filter papers pregated with N,N-dimethyl formamide as the stationary phase and cyclohexane saturated with N,N-dimethyl formamide as the mobile phase. Further identification of

TABLE II. THE FRACTIONS OF THE COLUMN CHROMATOGRAPHY OF 2,4-DINITROPHENYLHYDRAZONE

Fraction No.	Weight g.	2,4-dinitrophenylhydrazone	Methods of identification
1	trace	nonyl aldehyde	{m.p. 95°C (mixed m.p. 96°C)
2	0.10	2-hexenal	{m.p. 147°C (mixed m.p. 147°C)
3	0.42	{acetaldehyde <i>n</i> -propyl aldehyde <i>n</i> -butyraldehyde	$R_F=0.39$ $R_F=0.56$ $R_F=0.73$
4) 5) 6)	3.07	acetaldehyde	m.p. 167°C (mixed m.p. 167°C)
7 8	3.39	{acetaldehyde formaldehyde	$R_F=0.39$ $R_F=0.17$

some compounds isolated in pure state was attained by measuring the mixed melting points with authentic samples. The results of chromatography was shown in Table II.

The aqueous layer extracted from pentane solution as mentioned before was fractionally distilled under atmospheric pressure until the distillate was free from organic materials. Five fractions were collected and analyzed by gas chromatography after being dried with potassium carbonate. The composition of each fraction was shown in Table III.

TABLE III. THE COMPOSITION OF DISTILLED FRACTION OF AQUEOUS LAYER

Fraction No.	Volume ml.	Temp. (°C)	Compounds	Methods of identification
1	0.5	60~85	{ethyl alcohol <i>n</i> -propyl alcohol	GLC, $R_T=3.2$ $R_T=5.9$
2	1.3	85	{ <i>n</i> -propyl alcohol isobutyl alcohol <i>n</i> -butyl alcohol	$R_T=5.9$ $R_T=8.5$ $R_T=10.8$
3	1.0	85~91	<i>n</i> -butyl alcohol	$R_T=10.8$
4	1.7	91~98	{ <i>n</i> -butyl alcohol isoamyl alcohol	$R_T=10.8$ $R_T=17.5$
5	0.6	98~99	{ <i>n</i> -butyl alcohol isoamyl alcohol	$R_T=10.8$ $R_T=17.5$

A mixture of volatile acids recovered from aqueous sodium carbonate solution was esterified with ethyl alcohol and sulfuric acid which was followed by chromatography on silicic acid. Esters of isovaleric, caproic, benzoic acids, and an unknown fatty acid whose IR

3*) Column material, polyethylene glycol-celite; temperature, 130°C.

spectrum showed the presence of ethylenic linkage conjugated to carbonyl groups ($C=C$ 6.02 μ , $C=O$ 5.91 μ) were separated.

TABLE IV. COMPOUNDS IDENTIFIED FROM
"KOGYOKU" APPLES

Compound	g. in 21.7 kg. natural flavour essence
ethyl alcohol	0.3
<i>n</i> -propyl alcohol	1.0
<i>n</i> -butyl alcohol	8.4
isobutyl alcohol	0.7
<i>n</i> -amyl alcohol	0.1
(-)-2-methyl butanol	4.1
<i>n</i> -hexyl alcohol	2.5
hexen-3-ol-1	0.2
<i>n</i> -propyl acetate	0.2
<i>n</i> -butyl acetate	2.0
<i>n</i> -amyl acetate	0.6
<i>n</i> -hexyl acetate	0.2
ethyl caproate	+
formaldehyde	0.4
acetaldehyde	1.8
<i>n</i> -propionaldehyde	0.1
2-hexenal	0.1
nonyl aldehyde	+
<i>n</i> -butyraldehyde	+
isovaleric acid	0.2
caproic acid	0.8
benzoic acid	0.8
α,β -unsaturated acid	0.3

Results are summarized in Table IV.

The estimation of the quantity of each

component was attained by weighing the isolated fraction, as far as possible, and by calculating the peak area of gas chromatogram of further inseparable intermediate fraction.

It is rather difficult to separate 2-methyl butanol from isoamyl alcohol owing to close similarities of their boiling points and gas chromatographic retention time; isoamyl alcohol fraction of apple essential oil is evidently composed of almost pure 2-methyl butanol taking into account the value of its optical rotation, $[\alpha]_{18}^D = -5.42^{*4}$.

In general, much different aroma are noted among species of apples. Proportion of volatile constituents, especially, of esters in our specimen is rather different from the one reported regarding the foreign varieties. As shown in Table IV, the aroma of "KOGYOKU" apples is thought to be contributed mainly by the presence of esters of *n*-propyl, *n*-butyl, *n*-amyl and *n*-hexyl alcohols. The combination of these compounds listed above was found insufficient to reconstruct the fine aroma of "KOGYOKU" apple. This fact suggests the presence of some unidentified compounds which are essential to the aroma of fresh apples and the study of which will need far more expense and labour owing to their extremely minute quantities.

*4) Highest value reported, $[\alpha]_D^{20} = -5.90$.