CHROM. 5499

GAS CHROMATOGRAPHIC ANALYSIS OF FLAVOUR COMPONENTS WITH CORRELATION ISOTHERMAL RETENTION INDICES*

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(Received May 17th, 1971)

SUMMARY

A method was developed to identify compounds under temperature-programmed gas chromatography conditions with the correlation isothermal retention index. The method permits the use of tabulated index values without the use of standards. The correlation isothermal index was successfully applied for the identification under temperature-programmed gas chromatography conditions of some monocarbonyl compounds in volatile components of fresh salted salmon, sturgeon caviar and salmon roe.

INTRODUCTION

There are a number of correlation isothermal retention index formulae[1–4] for the identification of substances in linear temperature-programmed gas chromatography (TPGC).

In this study, the previously proposed[3] correlation isothermal index, $I_{T_0/\beta}$, has been successfully applied to the analysis of monocarbonyl compounds isolated from volatile components of such food products as fresh salted salmon, sturgeon caviar and salmon roe.

The data obtained permit us to recommend the use of $I_{T_0/\beta}$ for the identification of complex mixtures separated by TPGC. Since the method in question makes wide use of tabulated isothermal indices, it is easily applicable without the need for standard substances.

EXPERIMENTAL

Fresh salted salmon fillets and sturgeon caviar and salmon roe of high quality were used.

As described earlier[5], the volatile compounds were isolated *in vacuo*. The salmon had been previously chopped into small pieces. The distillate was treated with 2,4-dinitrophenylhydrazine and the resulting dinitrophenylhydrazone (DNP) derivatives of the monocarbonyl compounds were extracted with a mixture of benzene and hexane (1:1). The solvents had been preliminarily purified of trace amounts of carbonyls[6].

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Impurities in the DNPs were removed with ion-exchange resins[7]. Carbonyls were recovered from the DNPs[8] and 7–8 μl samples were injected into the GC column in ether solution with a Hamilton syringe.

Analyses were made with a Pye-Unicam 104 series Model 24 gas chromatograph equipped with a double-flame ionisation detector and a flow-rate controller. Glass columns (150 × 0.4 cm) were packed with Celite 545 (100–120 mesh) coated with 10 % of the stationary phases Apiezon-M (Ap-M) and polyethylene glycol adipate (PEGA). Isothermal analyses were carried out at 125°, the nitrogen flow rate being 60 ml/min, with standard C_8–C_{14} n-alkanes used for Ap-M and C_{11}–C_{17} n-alkanes for PEGA.

Retention index temperature gradients, $\partial I/\partial T$, of monocarbonyls were determined under isothermal conditions at 75°, 100°, 125° and 150°, some compounds also being investigated at 50°, 175° and 200°. Linear functions were obtained in each case, and Table I shows $\partial I/\partial T$ values.

TABLE I

TEMPERATURE GRADIENT[a] INDICES OF MONOCARBONYL COMPOUNDS

Stationary phase	*Alkan-2-ones,* C_5–C_{11}	*Alkanals,* C_5–C_{11}	*Alk-2-enals,* C_6, C_7, C_{10}	*Alk-2,4-dienals,* C_6, C_8
Apiezon-M	0.6	0.3	2.0	2.5
Polyethylene glycol adipate	8.0	8.0	13.0	15.0

[a] Temperature gradients are given for 10° intervals.

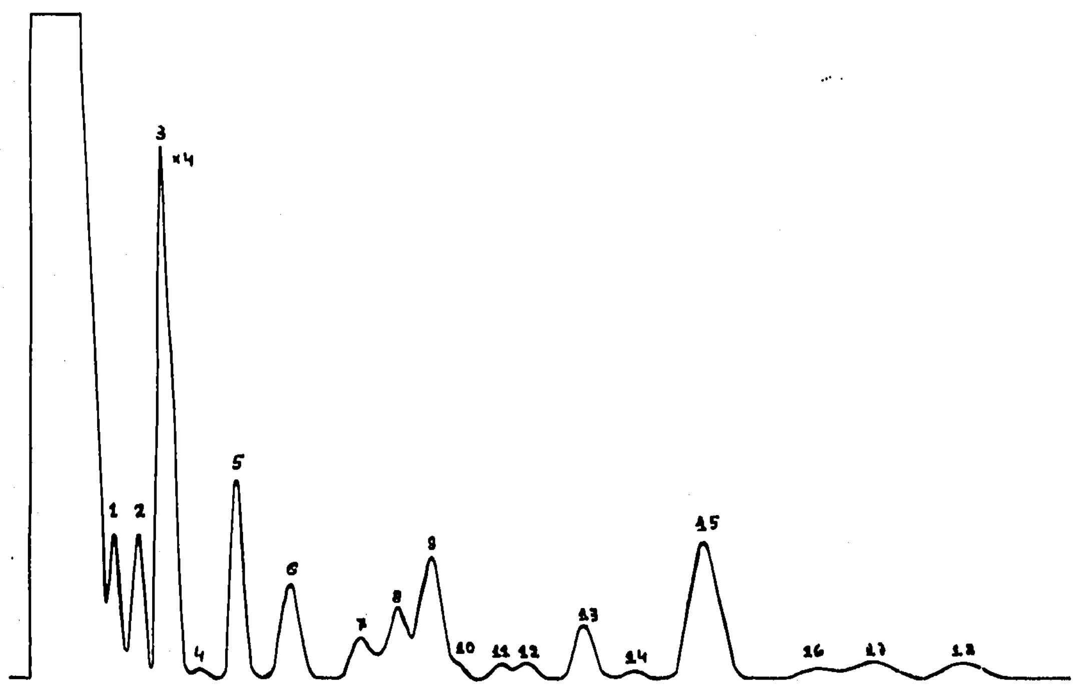

Fig. 1. Monocarbonyl compounds in volatiles of fresh salted salmon. Chromatogram made on the PEGA column at 125°. (For identification of the peaks, see text.)

We obtained chromatograms of volatile monocarbonyls of the above-mentioned food products and Fig. 1 shows one for the PEGA column. Table II gives index values for all the products using the same column.

TABLE II

RENTENTION INDICES OF MONOCARBONYL COMPOUNDS IN VOLATILE COMPONENTS OF SOME FOOD PRODUCTS ON THE PEGA COLUMN AT 125°

$I_{125°}^{PEGA}$				*Compound*
Salmon	*Sturgeon caviar*	*Salmon roe*	*I*[a] *of standard substance*	
1260	1252	1257	1257	Heptanal
			1259	Heptan-2-one
1313	1309	1316	1312	Hex-2-enal
			1357	Octanal
1358	1361	1355		
			1359	Octan-2-one
1416	1416	—	1411	Hept-2-enal
			1457	Nonanal
1460	1460	1461		
—			1458	Nonan-2-one
—	—	1476		Unidentified
1510	1514	1512	1510	Oct-2-enal
—	—	1529		Unidentified
			1555	Decanal
1560	1560	1559		
			1557	Decan-2-one
1583	1582	1584	1580	Hepta-2,4-dienal
1605	1615	1607	1609	Non-2-enal
1620	—	—		Unidentified
1637	—	—		Unidentified
1649	—	—		Unidentified
			1654	Undecanal
1654	1657	1651		
			1655	Undecan-2-one
1678	1674	1675	1674	Octa-2,4-dienal
1712	1702	1703	1708	Dec-2-enal
			1753	Dodecanal
1754	—	1758		
			1755	Dodecan-2-one
1780	—	—	1774	Nona-2,4-dienal

[a] Alk-2,4-dienal indices are published here for the first time; the other ones are taken from ref. 9.

The Ap-M column temperature increased from 75° to 200° at a rate of $\beta = 6°/\text{min}$, the flow rate being 60 ml/min. Standard C_6–C_{14} *n*-alkanes were used for the calculation of retention indices. The PEGA column temperature varied from 75° to

175°, with $\beta = 4$°/min, nitrogen flow rate 40 ml/min and C_8–C_{10} *n*-alkanes. Fig. 2 gives one of the chromatograms. Index values are listed in Tables III and IV.

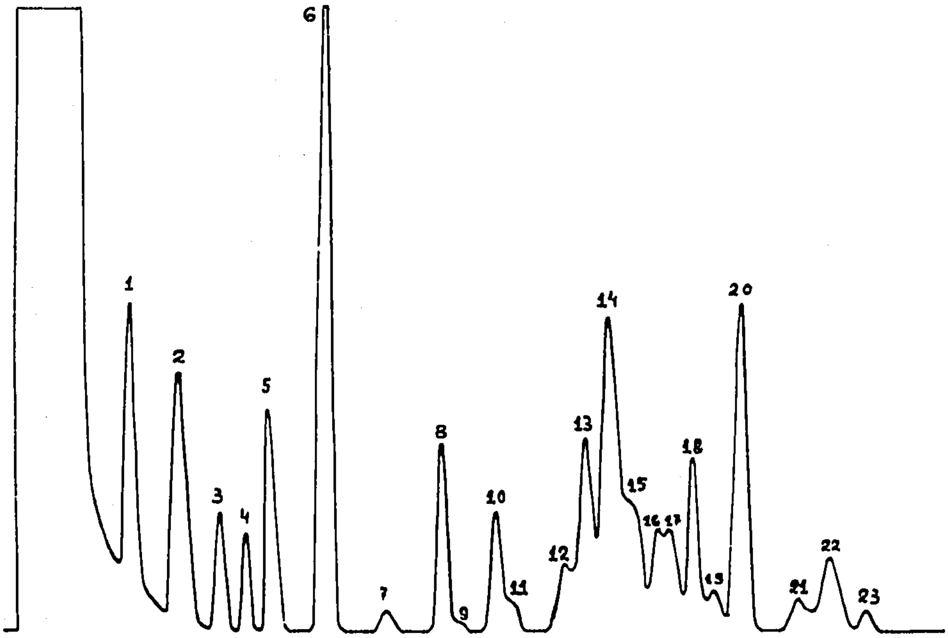

Fig. 2. Monocarbonyl compounds in volatiles of fresh salted salmon. Chromatogram made on the PEGA column under TPGC conditions. (For identification of the peaks, see text.)

DISCUSSION

We have previously proposed[9] a GC method of identifying substances in mixtures containing *n*-alkanals, *n*-alk-2-enals, *n*-alk-2,4-dienals, *n*-alkan-2-ones, symmetrical *n*-alkanones and a number of isomonocarbonyl compounds without the need for a standard to be used. Identification is effected with a system of equations showing the dependence of retention indices on the number of carbon atoms and the boiling points of substances.

The carbonyl compounds under investigation were isolated from flavour distillate and were subsequently analysed isothermally by this method and under TPGC conditions. Identification under TPGC conditions was accomplished with the aid of the isothermal indices we had obtained, taking into account the TPGC experimental conditions, *viz.* initial temperature, T_0, temperature programming rate, β, and gas flow rate, using the following equation[3]:

$$I_{T_0/\beta} = I_{T_0} + \frac{\beta \cdot T_x}{2} \cdot \frac{\partial I}{\partial T} \qquad (1)$$

where $\partial I/\partial T$ = index temperature gradient, T_x = retention time, and I_{T_0} = isothermal index at initial temperature T_0. Good agreement between the experimental I_{pr} indices of VAN DEN DOOL[10] and $I_{T_0/\beta}$ calculated from the isothermal data proves a correct identification (see Tables II and IV). The difference between $I_{T_0/\beta}$ and isothermal indices is small on the non-polar Ap-M column, while in the case of the PEGA column with its more pronounced polarity, this difference becomes considerable, for

TABLE III

RETENTION INDICES OF MONOCARBONYL COMPOUNDS IN VOLATILE COMPONENTS OF SOME FOOD PRODUCTS ON THE Ap-M COLUMN UNDER TPGC CONDITIONS

I_{pr}[a]				Compound
Salmon	*Sturgeon caviar*	*Salmon roe*	$I_{T_0/\beta}$[b]	
721	726	721	726	Pent-2-enal
753	747	755	751	Hexan-2-one
770	772	771	770	Hexanal
802	—	808	—	Unidentified
823	825	833	828	Hex-2-enal
847	—	—		Unidentified
852	856	—	852	Heptan-2-one
—	—	865		Unidentified
868	868	875	871	Heptanal
887	889	—	889	Hexa-2,4-dienal
906	—	904		Unidentified
927	—	930	931	Hept-2-enal
—	—	946		Unidentified
949	952	956	952	Octan-2-one
976	972	971	972	Octanal
987	987	987	990	Hepta-2,4-dienal
1011	—	1005		Unidentified
1019	—	1022		Unidentified
1033	1039	1028	1033	Oct-2-enal
—	—	1041		Unidentified
1051	1054	1052	1053	Nonan-2-one
—	—	1059		Unidentified
1072	1073	1076	1073	Nonanal
1084	1085	1094	1089	Octa-2,4-dienal
1101	—			Unidentified
1115	—	1122		Unidentified
1131	1136	1138	1134	Non-2-enal
1153	1156	1158	1154	Decan-2-one
—	—	1168		Unidentified
1175	1177	1180	1175	Decanal
1188	1196	1187	1190	Nona-2,4-dienal
1209	—	1201		Unidentified
1236	1239	1234	1236	Dec-2-enal
1259	1261	1257	1257	Undecan-2-one
1272	1280	1281	1276	Undecanal
1300	—	1301	1295	Deca-2,4-dienal
1318	—	1326		Unidentified
1339	—	1342	1338	Undec-2-enal
1361	—	1356	1358	Dodecan-2-one
1382	—	1375	1379	Dodecanal

[a] I_{pr} determined according to the equation of VAN DEN DOOL[10].
[b] $I_{T_0/\beta}$ calculated according to eqn. 1.

example thirty-four index units for *n*-heptanal (*cf.* Tables II and IV). Comparing the results of TPGC and isothermal analyses, we may, on the basis of eqn. 1, calculate the temperature gradient which, being a characteristic property[11], offers an additional identification criterion. Another advantage of comparing isothermal and TPGC chromatograms is that if two substances with identical indices, analysed isothermally, give one peak, the identification error on a TPGC chromatogram can be easily detected in the case that the $\partial I/\partial T$ values of the substances under investigation are different.

TABLE IV

RETENTION INDICES OF MONOCARBONYL COMPOUNDS IN VOLATILE COMPONENTS OF SOME FOOD PRODUCTS ON THE PEGA COLUMN UNDER TPGC CONDITIONS

Salmon		*Sturgeon caviar*		*Salmon roe*		*Compound*
I_{pr}[a]	$I_{T_0/\beta}$[b]	I_{pr}	$I_{T_0/\beta}$	I_{pr}	$I_{T_0/\beta}$	
1118	1121	1116	1120	1122	1122	Hexan-2-one; hexanal
1164	1169	1166	1168	1172	1170	Pent-2-enal
—		—		1201		Unidentified
1223	1225	1220	1224	1224	1225	Heptan-2-one; heptanal
1251		1245		1252		Unidentified
1270	1270	1274	1269	1271	1271	Hex-2-enal
—		—		1283		Unidentified
—		—		1311		Unidentified
1329	1329	1322	1326	1328	1329	Octan-2-one; octanal
—		1360		1362		Unidentified
1383	1378	1377	1376	1378	1379	Hept-2-enal
—		—		1389		Unidentified
1434	1434	1428	1430	1430	1434	Nonan-2-one; nonanal
—		—		1440		Unidentified
1454	1457	1455	1454	—		Hexa-2,4-dienal
1479	1480	1482	1478	1481	1481	Oct-2-enal
1502		1500		1508		Unidentified
1540	1539	1536	1537	1537	1539	Decan-2-one; decanal
1556	1553	1557	1551	1555	1554	Hepta-2,4-dienal
—		—		1574		Unidentified
1582	1584	1578	1580	1588	1585	Non-2-enal
1594		1592		—		Unidentified
1615		—		—		Unidentified
1625		—		1626		Unidentified
1647	1643	1646	1641	1646	1644	Undecan-2-one; undecanal
1658	1656	—				Octa-2,4-dienal
1699	1696	1695	1693	1703	1698	Dec-2-enal
—		—		1725		Unidentified
1749	1747	—		1752	1748	Dodecan-2-one; dodecanal
1770	1768					Nona-2,4-dienal

[a] I_{pr} determined according to the equation of VAN DEN DOOL[10].
[b] $I_{T_0/\beta}$ calculated according to eqn. 1; temperature gradients and isothermal indices are taken from Tables I and II, respectively.

For example, examination of isothermal and TPGC chromatograms of fresh salmon monocarbonyls on the PEGA column shows that peak 6 (Fig. 1) of the isothermal chromatogram corresponding to oct-2-enal does not belong to a single substance, but represents three compounds: oct-2-enal, hexa-2,4-dienal and an unidentified compound (peaks 9, 10, 11, Fig. 2).

Some volatile monocarbonyls of food products could not be identified. It was difficult to relate the peaks obtained on different columns to one substance owing to the small sizes of the peaks.

In our next communication, we hope to give a more detailed account of the carbonyl composition of the flavours of the food products dealt with in this paper.

Our results show that the method proposed here makes it possible to use for identification isothermal indices from tables easily available in many recent publications.

Tabulated indices for identication in TPGC can be successfully applied, provided that satisfactory reproducibility of chromatographic columns is possible. In this connection, a method characterising a column in some definite terms acquires particular importance, for example Rohrschneider constants.

Our monocarbonyl isothermal indices obtained on Celite 545 with 10% Ap-M differed insignificantly from the corresponding values in McReynolds' tables, despite the use of Chromosorb W as a support. The three years' research in our laboratory with different types of glass columns containing Celite 545 coated with PEGA have shown the accuracy of reproduction for isothermal indices to be about three units. This is sufficient for identification in TPGC.

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