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Studies on the Volatile Components of Peated Malt. II.[1) Identification of Neutral Components

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The neutral fraction obtained from the volatile components of peated malt was examined by mass spectrometry combined with gas chromatography. Eight kinds of aldehyde, ten kinds of alcohol, seven kinds of fatty acid ester, and twelve kinds of alkane were identified. Isovaleraldehyde, acetaldehyde, and isobutylaldehyde were major components, and their relative peak area was about 60% in all. Formaldehyde, furfural, propylaldehyde, and valeraldehyde were minor components. An abundance of alkanes appeared in the neutral fraction. The major components identified as fatty acid ethyl esters were hexadecenoate, oleate, and linoleate. Contribution of these compounds to the flavor of peated malt is discussed.

Introduction

Aroma components of alcoholic beverages produced by fermentation of different raw materials have been reported.[3)] In view of nonenzymic formation of aromatic components, the behavior of carbonyl compounds produced by cooking has been discussed and a number of volatile carbonyl compounds related to flavor were identified.[4)] It is well known that some carbonyl compounds derived from cooking process play an important role as desirable flavor in prepared food.[5)] Qualitative aspects of volatile carbonyl compounds that might be associated with roast flavor has also been reported by many investigators.[6)] On the other hand, several carbonyl compounds in alcoholic beverages seem to be not only produced from fermentation process, but also derived from raw materials by cooking. Aliphatic alcohols of low molecular weight are one of the important components which contribute to the aroma of fruits.[7)] Most of the aliphatic alcohols in brewing products was regarded as of major importance of fusel oil,[8)] and should also be evaluated as compounds which participate in ester formation. In general, malt dried over a slow-buring peat fire was used for whisky brewing as raw materials. Thus, the constituents in peated malt are expected to contribute to whisky flavor.

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1) *a*) Part I: M. Deki and M. Yoshimura, *Chem. Pharm. Bull.* (Tokyo), 22, 1748 (1974). *b*) A part of this work was presented at the 126th Annual Meeting of the Nishi Nippon Branch of the Agricultural Chemical Society of Japan, Nagasaki, October, 1971.

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3) *a*) Y. Hirose, M. Ogawa and Y. Kusuda, *Agr. Biol. Chem.* (Tokyo), **26**, 526 (1962); *b*) E. Matsumura, T. Kuramae, M. Deki, A. Oobayashi and M. Kanie, *Nippon Zyozo Kyokai Shi*, **68**, 699 (1973).

4) *a*) Y. Linko, J.A. Johnson and B.S. Miller, *Cereal Chem.*, **39**, 468 (1962); *b*) E. Collin, *J. Agr. Food Chem.*, **19**, 533 (1971).

5) *a*) W. Fiddler, R.C. Doerr, A.E. Wasserman and J.M. Salay, *Agr. Food Chem.*, **14**, 659 (1966); *b*) A.E. Wasserman, *J. Food Sci.*, **31**, 1005 (1966); *c*) R.H. Walter and I.S. Fagerson, *J. Food Sci.*, **33**, 294 (1968).

6) *a*) D.F. Brown, V.J. Senn, J.E. Sanley and F.G. Dollar, *J. Agr. Food Chem.*, **20**, 700 (1972); *b*) D.F. Brown, V.J. Senn, F.G. Dollar and L.A. Goldblatt, *J. Am. Chem. Soc.*, **50**, 16 (1973).

7) E.L. Wick, T. Yamanishi, A. Kobayashi, S. Valenzuela and P. Issenberg, *J. Agr. Food Chem.*, **17**, 751 (1969).

8) J.H. Kahn, E.G. LaRoe and H.A. Conner, *J. Food Sci.*, **33**, 395 (1968).

This paper deals with the separation and identification of neutral components from the distillate of peated malt using mass spectrometry combined with gas chromatography.

Experimental

Materials——Heavily peated malt and nonpeated malt manufactured in England were used in this experiment.

Collection of Carbonyl Compounds from Head Space Vapor——About 500 g of peated malt was subjected to steam distillation in a current of N_2 gas. The vapor was absorbed in a solution of 2,4-dinitrophenylhydrazine (2 g/liter of 2N HCl solution). The precipitate formed was collected by filtration, washed with 2N HCl solution and water, and dried over silica gel in a vacuum desiccator.

Collection of Carbonyl Compounds from Distillate——To 500 ml of the distillate obtained by the above distillation, 100 ml of 12N HCl solution was added, followed by 100 ml of a saturated solution of 2,4-dinitrophenylhydrazine in 2N HCl solution, and the mixture was allowed to stand at room temperature for 12 hr. The precipitate formed was collected by filtration, washed with 2N HCl solution and H_2O, and dried over silica gel in a vacuum desiccator. For the gas chromatography and mass spectrometry combined with gas chromatography, 2,4-dinitrophenylhydrazone of carbonyl compound was dissolved in $CHCl_3$ to a 0.5% solution.

Preparation of Neutral Components——Neutral compounds were isolated from the distillate using a procedure previously described.[1a)]

Conditions of Gas Chromatography and Mass Spectrometry Combined with Gas Chromatography——Shimadzu GC 5APF gas chromatograph equipped with a flame ionization detector was employed in this experiment. Two columns packed with polyethylene glycol (PEG) 20 M (5%) and OV-101 (3%) on 80 to 100 mesh Chromosorb GAW–HMDS were employed. Temperature in column oven was programmed under the conditions given in Fig. 1 and 2. Conditions of gas chromatographic analysis for mass spectrometry combined with gas chromatography were the same as above. The mass spectrometric analysis was carried out on an LKB 9000 connected with the gas chromatograph. Mass spectra were run at an ionization voltage of 70 eV, accelerating voltage of 3.5 kV, trap current of 60 μA, and separator temperature of 250°.

Result and Discussion

Identification of Volatile Carbonyl Compounds

Direct analysis of 2,4-dinitrophenylhydrazone of carbonyl compounds in peated malt was performed by mass spectrometry combined with gas chromatography. The gas chromatograms of 2,4-dinitrophenylhydrazone obtained from head space and distillate fraction are shown in Fig. 1. Both fractions showed somewhat similar gas chromatogram, but in the head space fraction, some of the minor components were not observed.

The major peaks in both fractions are peaks 4, 6, and 8, but the relative peak area of peak 8 in the distillate fraction is about twice more than that of the head space fraction. Halvarson[9)] reported that 2,4-dinitrophenylhydrazone of formaldehyde could not be detected by the method of combined regeneration gas chromatography–mass spectrometry. However, it is clear that 2,4-dinitrophenylhydrazone of formaldehyde can be identified by direct analysis with mass spectrometry combined with gas chromatography. For example, peak 2 was identified as 2,4-dinitrophenylhydrazone of formaldehyde. The mass spectrum which shows the molecular ion at m/e 210 and fragment ions at m/e 188, 142, and 122, was compatible with that of authentic 2,4-dinitrophenylhydrazone of formaldehyde.

Peaks 3 and 4 were tentatively identified as two isomeric 2,4-dinitrophenylhydrazone of acetaldehyde with a molecular weight of 224. The mass spectra of these two peaks were similar to that of authentic 2,4-dinitrophenylhydrazone of acetaldehyde. However, there are slight differences in mass spectrum between these two peaks. For example, in the spectrum of peak 3 the fragment peaks at m/e 153, 154, and 183 were predominant than that of peak 4. Contrarily, in the case of peak 4 the fragment peak at m/e 152 was more predominant compared with that of peak 3. Peaks 6 and 7 were identified as 2,4-dinitrophenylhydrazone of two

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9) H. Halvarson, *J. Chromatogr.*, **66**, 35 (1972).

isomer of butylaldehyde with a molecular weight of 252; isobutylaldehyde (peak 6) and butylaldehyde (peak 7). Their mass spectra and retention indices were in close agreement with those of the authentic compounds. On the basis of mass spectra and retention indices, peaks 8 and 9 were identified as 2,4-dinitrophenylhydrazone of isovaleraldehyde and valeraldehyde, respectively. Peak 10 was identified as 2,4-dinitrophenylhydrazone of furfural, and its mass spectrum was very similar to that of authentic 2,4-dinitrophenylhydrazone of furfural. The mass data and relative peak area of 2,4-dinitrophenylhydrazone identified are listed in Table I.

It is clear that the volatile carbonyl compounds are main component which indicate the characteristic aroma in head space vapor, since caramel–like aroma produced on distillation of peated malt disappeared after trapped as 2,4-dinitrophenylhydrazone. Formal-

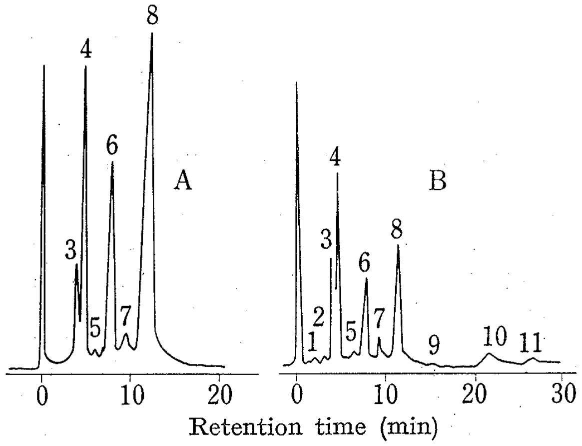

Fig. 1. Gas Chromatograms of 2,4-Dinitrophenylhydrazone of Carbonyl Compounds obtained from Peated Malt

A: head space fraction, B: distillate fraction
GC conditions: column 3% OV-101 on Chromosorb GAW–HMDS, column temp. 140° to 300°, 5°/min, injection temp. 320°, He flow rate 60 ml/min

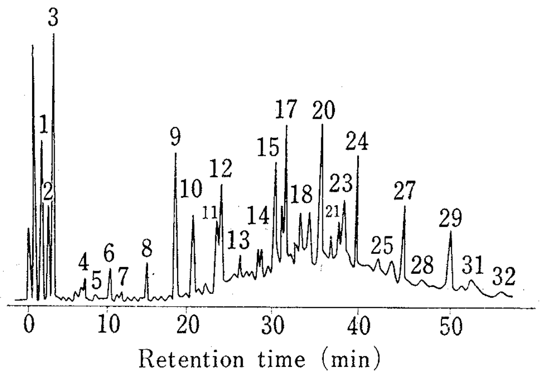

Fig. 2. Gas Chromatogram of Neutral Fraction obtained from the Distillate of Peated Malt

GC conditions: column 5% PEG-20M on Chromosorb GAW, column temp. 80° to 250°, 5°/min, injection temp. 280°, He flow rate 60 ml/min

Table I. Mass Spectra of 2,4-Dinitrophenylhydrazone of Carbonyl Compounds identified in the Distillate obtained from peated Malt

Peak No.a)	Compound	Rt^{+} (min)	Peak area^{++} (%)	m/e (relative intensity %)
2	formaldehyde	1.2	1.4	M$^+$ 210(50), 63(100), 91(85), 79(85), 183(70), 41(50), 51(45), 148(20)
3	acetaldehyde	1.6	16.6	M$^+$ 224(90), 79(100), 63(50), 41(46), 153(30), 122(25), 91(22), 183(20)
4	acetaldehyde	1.9	23.3	M$^+$ 224(90), 79(100), 63(50), 41(50), 180(20), 152(18), 122(18), 91(15)
5	propylaldehyde	3.0	4.0	M$^+$ 238(100), 79(90), 41(90), 56(70), 59(60), 39(57), 91(50), 152(50)
6	isobutylaldehyde	3.7	13.2	M$^+$ 252(100), 41(95), 79(60), 152(60), 122(56), 55(50), 39(48), 75(45)
7	butylaldehyde	4.6	7.1	M$^+$ 252(100), 41(98), 43(98), 79(85), 152(75), 122(70), 55(70), 39(60)
8	isovaleraldehyde	5.8	22.7	M$^+$ 266(45), 41(100), 55(56), 39(50), 69(45), 57(46), 43(45), 152(40)
9	valeraldehyde	7.2	0.1	M$^+$ 266(56), 41(100), 69(55), 55(46), 39(45), 75(40), 63(38), 122(35)
10	furfural	10.9	7.5	M$^+$ 276(100), 97(80), 95(75), 39(85), 53(74), 65(60), 122(45), 155(40)

a) Peak numbers and GC conditions are the same as cited in Fig. 1, except for column temperature at 190°.
+: retention time. ++: relative peak area.

dehyde, propylaldehyde, butylaldehyde, and valeraldehyde are minor components in the distillate of peated malt. It is assumed that contribution of these minor components to the aroma of the distillate of peated malt is not so much. There is no quantitative difference in carbonyl compounds between the distillate and head space fractions. These volatile carbonyl compounds identified from peated malt do not originate from peat smoke but resulted from malt by cooking.

Identification of Neutral Components Other than Volatile Carbonyl Compounds

The neutral fraction obtained from total volatile condensate of peated malt has a weak vanilla-like aroma. A typical gas chromatogram of the neutral fraction is shown in Fig. 2.

Peaks 1, 2, 16, 23, 24, 29, and 31 show a typical fragmentation characteristics of fatty acid esters. The mass spectra of peaks 1 and 2 were in close agreement with those of the authentic methyl acetate (peak 1) and ethyl acetate (peak 2), respectively. Both peaks 16 and 23 show fragment peaks of M^+-29, M^+-43, and M^+-45, and the most intense peak is rearrangement ion at *m/e* 88 which indicates characteristic of fatty acid ethyl ester. The mass spectra and retention indices of these peaks agreed with those of authentic ethyl myristate (peak 16) and ethyl palmitate (peak 23), respectively. Peaks 24, 29, and 31 were identified as ethyl hexadecenoate (peak 24), ethyl oleate (peak 29), and ethyl linoleate (peak 32), respectively. The mass spectra and retention indices of these compounds were in close agreement with those of the authentic componds. Hexadecenoate, oleate, and linoleate were the main components of all fatty acid ethyl esters in neutral fraction, whereas myristate and palmitate appeared as quantitatively minor components. However, these compounds have not been reported to be common constituents of raw malt, although many kinds of fatty acid ethyl esters were found in whisky[10] but not in condensate of wood smoke. Thus, it is worth nothing that some of the fatty acid ethyl esters are found in raw malt.

Many saturated hydrocarbons were found in the neutral fraction. The compounds identified are listed in Table II. It is assumed that these long-chain hydrocarbons originated from the waxes in malt. Based on their flavor, the hydrocarbons did not appear to offer a significant contribution to the characteristic peated malt aroma.

TABLE II. Hydrocarbons identified in the Neutral Fraction obtained from the Distillate of Peated Malt

Compound	Peak No.[a]	Molecular ion (*m/e*)	Compound	Peak No.[a]	Molecular ion (*m/e*)
$C_{14}H_{30}$	6	198	$C_{21}H_{44}$	18	296
$C_{15}H_{32}$	7	212	$C_{22}H_{46}$	22	310
$C_{16}H_{34}$	8	226	$C_{23}H_{48}$	25	324
$C_{17}H_{36}$	9	240	$C_{24}H_{50}$	27	338
$C_{18}H_{38}$	10	254	$C_{25}H_{52}$	30	352
$C_{19}H_{40}$	13	268	$C_{27}H_{56}$	32	380
$C_{20}H_{42}$	15	282			

a) Peak numbers are the same as in Fig. 2.

Alcohols in neutral fraction were converted to trimethylsilyl derivatives, and identified by mass spectrometry combined with gas chromatography. The trimethylsilyl ethers of alcohols produced distinct mass spectra, and showed several intense rearrangement ion such as *m/e* 73, 89, and 103, which have been correlated with the trimethylsilyl ether structure. In general, abundance of molecular ion peak of silyl ethers is very weak or absent, but an intense peak resulting from the loss of one of the four methyl groups appears 15 mass unit

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10) K. Nishimura and M. Masuda, *J. Food Sci.*, **36**, 819 (1971).

below the parent mass.[11] Furthermore, the silyl ether of polyalcohols show an intense peak at *m/e* 147 corresponding to the rearrangement ion of disiloxonium ion. Thus, if the ion at *m/e* 147 is absent in the mass spectrum of silyl ethers, it can be considered that the original compound has only one OH group in the molecular formula.[12] Gas chromatogram of silylated neutral fraction is shown in Fig. 3.

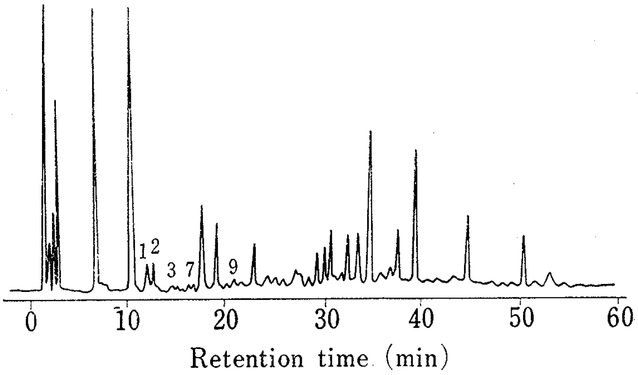

Fig. 3. Gas Chromatogram of Trimethylsilyl Derivatives of Neutral Fraction obtained from the Distillate of peated Malt

GC conditions are the same as in Fig. 2. The silylation of neutral fraction was constructed as follows: 0.1 ml of N,O-bis (trimethylsilyl) acetamide was added to the same volume of neutral fraction, and the tube sealed. The reaction mixture was shaken for 1 min and allowed to stand for 1 hr.

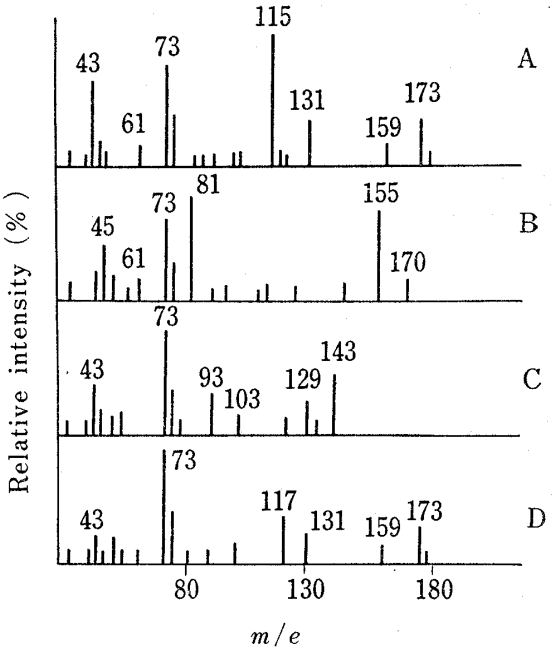

Fig. 4. Mass Spectra of silylated Compounds in Neutral Fraction

A: peak 1, B: peak 3, C: peak 7, D: peak 9. Peak numbers are the same as in Fig. 3.

Both peaks 1 and 9 showed the same fragment ion at *m/e* 173 which was the highest mass number on the spectrum. Thus the molecular weight of silyl ether was 188. In the case of peak 1, the most intense peak was the fragment ion at *m/e* 115 resulting from the loss of Me_3Si from molecular ion, and the presence of an intense peak at *m/e* 131 indicated the presence of a functional carbon at 3-position. The mass spectrum of peak 1 was in agreement with that of the authentic 3-heptanol trimethylsilyl ether. In the case of peak 9, however, rearrangement ion at *m/e* 73 was the base peak. It was assumed that the more intense peak at *m/e* 117 resulted from a break at the functional carbon at 2-position. Peak 9 was identified as 2-heptanol trimethylsilyl ether, and its mass spectrum was in close agreement with that of the authentic compound. Peak 7 was tentatively identified as cis-pent-2-en-1-ol trimethylsilyl ether. In the mass spectrum of peak 7, the fragment ion at *m/e* 143 (M^+-15) was the highest mass peak. The molecular weight of silyl ether was 158 and, therefore, molecular weight of the original compound is 86. Also typical fragment ions at *m/e* 129 and 103 were observed. The mass spectrum of peak 7 was compatible with that of the authentic compound. The mass spectra of these peaks are shown in Fig. 4.

2-Methyl-1-pentanol, 1-heptanol, nonanol, 2-hydroxy-5-methylcyclopent-2-en-1-one, 2-phenylethanol, and 3-phenylpropanol were tentatively identified from their mass spectra.[13]

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11) A.G. Sharkey, Jr., R.A. Friedel and S.H. Langer, *Anal. Chem.*, 29, 770 (1957).

12) P. Capella and C.M. Zorzut, *Anal. Chem.*, 40, 1458 (1968).

13) *a*) A.G. Sharkey, Jr., J.L. Shulta and R.A. Friedel, *Anal. Chem.*, 31, 87 (1959); *b*) E.M. Emery, *Anal. Chem.*, 32, 1495 (1960).

The flavor properties of these alcohols were evaluated as flavor associated with aroma of roast and cooked food. Most of these neutral compounds identified from peated malt have a distinctive aroma which may contribute to the characteristic flavor of alcoholic beverages.

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