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Studies on the Volatile Components of Peated Malt. III.[1a] Identification of Acidic and Basic Components[1b]

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Basic and acidic fractions of the distillate of peated malt were analysed by mass spectrometry combined with gas chromatography. Six pyrazines, two pyrroles, 14 fatty acids, 2-furoic acid, phenylacetic acid, benzoic acid, and cinnamic acid were identified. The major components in basic fraction were 2,5-dimethylpyrazine and 2-methyl-5-acetylpyrazine. The basic components identified in nonpeated malt were qualitatively similar to those of peated malt. Palmitic, stearic, and oleic acids were predominant in acidic fraction obtained from ether extract of peated malt. Benzoic acid, cinnamic acid, phenylacetic acid, and furoic acid were isolated as minor components.

Introduction

It has been reported by some investigators that the phenolic compounds in malt contributed to the aroma of whisky,[3] but the components other than phenolic compounds have not yet been discussed. It is assumed that the phenolic compounds are directly related to the smoke flavor of whisky. However, as evidenced from investigation of wood smoke constituents, participation of carbonyl, acidic and pyrazine compounds is necessary to obtain an agreeable flavor.[4] A number of volatile fatty acids have been found in thermal degradation products of carbohydrate.[5] Based on its flavor, these fatty acids have scarcely been related to aroma directly but, fatty acid esters have been implicated with the characteristic flavor of foodstuff.[6] On the other hand, some of the basic compounds have become of major interest as roast aroma in roasted foods. In fact, a number of alkylpyrazines and other heterocyclic nitrogen-containing compounds have been found in prepared foods, and their role in roast aroma has been investigated.[7] Many kinds of pyrazine compounds have been isolated from whisky. Nishimura, *et al.*[8] have shown that these pyrazine compounds were produced from cooked grain during the mashing process.

The present study was made to determine the acidic and basic components in the distillate of peated malt, and also to compare the acidic compounds in peated malt and that of nonpeated malt.

1) *a*) Part II: M. Deki and M. Yoshimura, *Chem. Pharm. Bull.* (Tokyo), **22**, 1754 (1974). *b*) A part of this work was presented at the 46th Annual Meeting of the Agricultural Chemical Society of Japan, Tokyo, April, 1971.
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3) *a*) C. Macfarlane, *J. Inst. Brewing*, **74**, 272 (1968); *b*) R.D. Steinke and M.C. Paulson, *Agr. Food Chem.*, **12**, 381 (1964).
4) *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*) E. Collins, *J. Agr. Food Chem.*, **19**, 533 (1971).
5) R.H. Walter and I.S. Fagerson, *J. Agr. Food Chem.*, **19**, 533 (1971).
6) *a*) L. Nykanen, E. Puputti and H. Suomalin, *J. Food Sci.*, **33**, 88 (1968); *b*) E. Matsumura, T. Kuramae, M. Deki, A. Oobayashi and M. Kanie, *Nippon Zyozo Kyokai Shi*, **68**, 699 (1973).
7) P.E. Koehler, M.E. Mason and G.V. Odeii, *J. Food Sci.*, **36**, 816 (1971).
8) K. Nishimura and M. Masuda, *J. Food Sci.*, **36**, 819 (1971).

Experimental

Materials——The heavily peated and nonpeated malts manufactured in England were used in this experiment.

Preparation of Acidic and Basic Fractions from Distillate of Peated Malt——The peated malt was steam distilled by the usual method. The distillate was fractionated into acidic and basic fractions using the procedure described previously.[9] The acidic fraction was converted into methyl esters with diazomethane, and analyzed by gas chromatography. Both acidic and basic components were identified by mass spectrometry combined with gas chromatography. Tentative identification was made by comparing the retention times of unknown compounds with those of authentic compounds.

Conditions of Gas Chromatography and Mass Spectrometry combined with Gas Chromatography——Analytical conditions for gas chromatography and mass spectrometry were the same as those previously described.[9] The methylated fatty acids were separated on a glass column containing 25% diethylene glycol succinate (DEGS) on 80—100 mesh Chromosorb GAW.

Result and Discussion

Identification of Basic Components

Basic fraction obtained from steam distillate had an unpleasant amine-like flavor. A typical gas chromatogram of basic fraction is shown in Fig. 1. Peaks 1, 2, 4, 7, and 8 are the major components of the basic fraction.

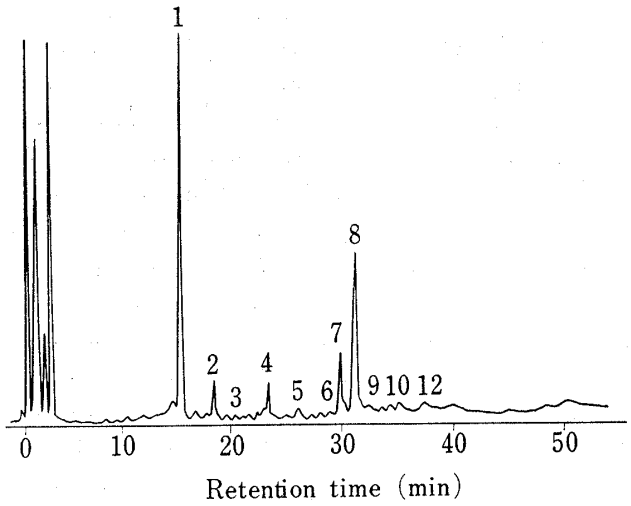

Fig. 1. Gas Chromatogram of Basic Fraction Obtained from the Distillate of Peated Malt

GC conditions: column packed with 5% PEG 20M on Chromosorb GAW, temperature in column oven 80—250° programmed at 5°/min, He flow rate 60 ml/min

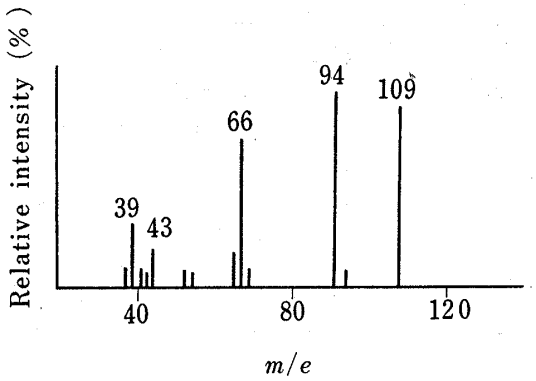

Fig. 2. Mass Spectrum of Peak 10 in Basic Fraction obtained from the Distillate of Peated Malt

Peak number is the same as in Fig. 1.

A mass spectrum of peak 1 showed typical fragment peak at *m/e* 42 due to the acetonitrile ion ($CH_3C{\equiv}NH$), and fragment pattern and retention index were compatible with those of authentic 2,5-dimethylpyrazine. Peak 2 was identified as 2-ethylpyrazine. The mass spectrum and retention index agreed with those of the authentic compounds. Peak 4 was identified as 2,3,5-trimethylpyrazine, and its mass spectrum agreed with that of authentic compound. Peak 5 was found to be 2-propylpyrazine, and its mass spectrum and retention index were compatible with those of the authentic compound. Peak 6 was identified as 2,6-diethylpyrazine, and its mass spectrum and retention index were in close agreement with those of the authentic compound. Peak 8 was tentatively identified as 2-methyl-5-acetylpyrazine, and its mass spectrum and retention index were compatible with those of the authentic compound. Peak 9 was found to be 1-furfurylpyrrole, and its mass spectrum and retention index

9) M. Deki and M. Yoshimura, *Chem. Pharm. Bull.* (Tokyo), **22**, 1748 (1974).

agreed with those of the authentic compound. Peak 10 was identified as 2-acetylpyrrole. The mass spectrum of peak 10 is shown in Fig. 2. The fragmentation mode was compatible with that of an authentic compound. The mass data of these peaks are summarized in Table I.

Table I. Compounds identified in Basic Fraction of the Distillate of peated Malt

Peak No.[a]	Compound	*m/e* (Relative intensity of main peaks %)
1	2,5-dimethylpyrazine	M^+ 108(100), 81(9), 42(76), 40(32), 39(42)
2	2-ethylpyrazine	M^+ 108(80), 107(100), 80(24), 53(22), 39(28)
4	2,3,5-trimethylpyrazine	M^+ 122(82), 81(18), 42(100), 39(28), 27(15)
5	2-propylpyrazine	M^+ 122(17), 107(23), 94(100), 53(8), 39(15)
6	2,6-diethylpyrazine	M^+ 136(58), 135(100), 108(15), 53(11), 39(17)
7	2,3,5-triethylpyrazine	M^+ 164(100), 163(50), 149(75), 56(40), 39(45)
8	2-methyl-5-acetylpyrazine	M^+ 136(100), 121(25), 94(98), 43(20), 39(65)
9	1-furfurylpyrrole	M^+ 147(95), 81(100), 53(55), 39(50), 27(45)
10	2-acetylpyrrole	M^+ 109(95), 94(100), 66(80), 43(20), 39(40)

a) Peak numbers are the same as in Fig. 1.

Mason, *et al.*[10] found pyrrole compounds in roasted peanuts, and Hodge[11] discussed the role of pyrrole compounds in roasted foods. Nishimura, *et al.*[8] also found pyrazine and quinoline in barley malt kilned with peat fire, and suggested that these pyrazine and quinoline derivatives in whisky came from peat smoke. As a result of model system studies,[12] alkylpyrazine formation has been correlated to the reaction of amino acid and carbohydrate in Maillard reaction. Pyrrole compounds identified were minor components. A number of pyrazine compounds such as 2-ethylpyrazine, 2-ethyl-6-methylpyrazine, and 2,3,5-trimethylpyrazine were also identified in the basic fraction from roasted malt. However, pyrazine compounds found in peated malt were not detected in peat smoke condensate. Furthermore, pyrazine compounds similar to those in the peated malt were also found in nonpeated malt. These facts imply that alkylpyrazine compounds in peated malt resulted from the raw malt during steam distillation, and the probability that pyrazine compounds resulting from peat smoke are responsible for aroma of peated malt is rather low. However, pyrazine compounds identified in peated malt seem to participate in the aroma of alcoholic bevarages with characteristic flavor, because several pyrazine compounds have been found in whisky.

Identification of Acidic Components

Quantitative and qualitative aspects of short-chain fatty acids found in cooked food that might be associated with flavor have been reported by several investigators.[13] In fact some of the acidic compounds such as formic, acetic, pyruvic, and methylfuroic acids have been isolated in the condensate obtained by thermal degradation of carbohydrate.[14] Furthermore, a number of short-chain fatty acids (C_2 to C_7), phenylacetic acid, and dicarboxylic acids have been found in roasted food, and formic acid, propionic acid, and unsaturated fatty acids have been isolated from wood smoke condensate. These acidic compounds seem to have a role as an intermediate of aroma formed during the cooking process. The gas chromatogram of the methylated acidic fraction obtained from steam distillate of peated malt is shown in Fig. 3.

10) M.E. Mason and B. Johnson, *J. Agr. Food Chem.*, **14**, 454 (1966).
11) J.E. Hodge, *J. Agr. Food Chem.*, **1**, 978 (1953).
12) P.E. Koehler, M.E. Mason and J.A. Newell, *J. Agr. Food Chem.*, **17**, 393 (1969).
13) I.R. Hunter, H. NG and J.W. Pence, *J. Food Sci.*, **26**, 578 (1961).
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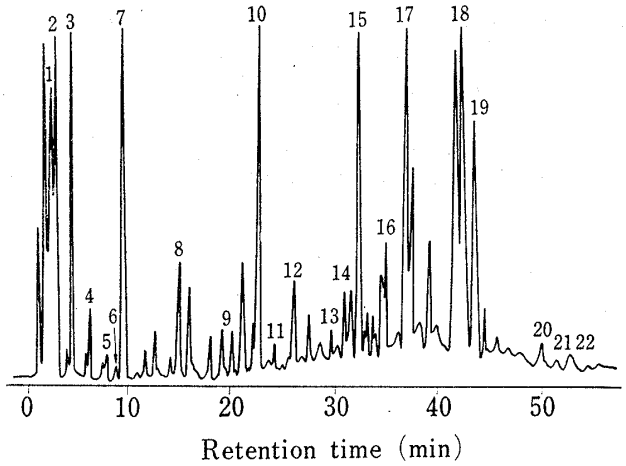

Fig. 3. Gas Chromatogram of Acidic Fraction obtained from the Distillate of Peated Malt

GC conditions: column packed with 25% DEGS on Chromosorb GAW, temperature in column oven 170°, He flow rate 60 ml/min Peaks 1. acetic acid, 2. propionic acid, 4. isobutyric acid, 5. isovaleric acid, 6. valeric acid, 7. isocaproic acid, 10. caprylic acid, 12. capric acid, 13. 2-furoic acid, 14. benzoic acid, 15. lauric acid, 16. phenylacetic acid, 17. myristic acid, 18. cinnamic acid, 19. palmitic acid, 20. stearic acid, 21. oleic acid, 22. linoleic acid

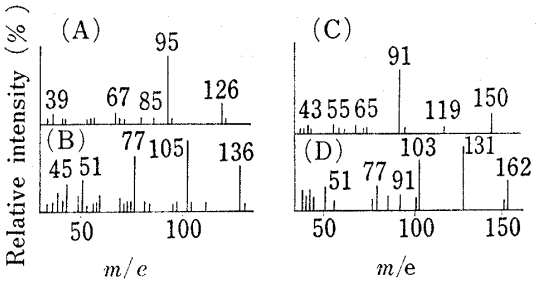

Fig. 4. Mass Spectra of Methylated Compounds in Acidic Fraction obtained from the Distillate of Peated Malt

(A) peak 13, (B) peak 14, (C) peak 16, (D) peak 18. Peak numbers are the same as in Fig. 3.

The major components of fatty acids identified are acetic acid (peak 1), propionic acid (peak 2), 2-methylpropionic acid (peak 4), 3-methylbutyric acid (peak 5), valeric acid (peak 6), and 4-methylvaleric acid (peak 7). In the short-chain fatty acids, 4-methylvaleric acid was the most abundant, being about 30% of total components of acidic fraction.

From the results of mass spectrometry, peak 8, 9, 10, 11, 12, 15, 17, 19, and 20 were identified as fatty acids of carbon number C_6, C_7, C_8, C_9, C_{10}, C_{12}, C_{14}, C_{16}, and C_{18}, respectively. Peaks 21 and 22 were identified as oleic and linoleic acids, respectively. Their mass spectra and retention indices were in close agreement with those of the authentic compounds. As shown in Fig. 3, relative amount of unsaturated higher carbon fatty acids was much lower than saturated fatty acids. These fatty acids identified in peated malt would be expected to be formed by the thermal degradation of glyceride in malt, and by the enzymic hydrolysis of glyceride in barley.

The mass spectrum of peak 13 is shown in Fig. 4 (A). The spectrum revealed more intense molecular ion at *m/e* 126, and the fragment ion at *m/e* 95 resulting from the loss of OCH_3 from the molecular ion was base peak. Thus the molecular weight of original acid is 112. The mass spectrum was in close agreement with that of authentic methyl 2-furoate. 2-Furoic acid has been found in the condensate of thermal degradation products of glucose, and its methyl ester was noted as characteristic flavor of roasted food. Peak 14 was identified as methyl benzoate (Fig. 4 (B)). The mass spectrum was compatible with that of the authentic compound. Peak 16 was identified as methyl phenylacetate (Fig. 4 (C)), and its mass spectrum closely agreed with that of the authentic compound. Peak 18 showed a typical mass spectrum of aromatic acid esters (Fig. 4 (D)). The presence of molecular ion at *m/e* 162 and fragment ions at *m/e* 132 (M^+-OCH_3) and *m/e* 103 ($M^+-COOCH_3$) were compatible with the mass data of the authentic methyl cinnamate. Since cinnamic acid was converted into a favorable flavor by fermentation of yeast and mold, these aromatic acids identified in peated malt seem to be precursors for producing agreeable flavor of whisky. In comparing the acids in peated and nonpeated malt, similarity in composition of acid fraction is remarkable. Concentration of higher carbon-chain fatty acids such as palmitic, stearic, and oleic acids in the acidic fraction obtained from the condensate of steam distillation was lower than those of acidic fraction obtained from ether extract of malt. Although unsaturated fatty acids act as off-

flavor in brewing products, esters of these fatty acids have a characteristic flavor that contributes to an agreeable flavor of alcoholic bevarages. Thus the fatty acids contained in raw malt were considered to have some effect on the aroma of whisky and beer.

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