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Studies on the Volatile Components of Peated Malt. I. Identification of Phenolic Compounds

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The total phenol content in peated malt varied in a range of 2 to 15 ppm. The high content of total phenols in heavily peated malt was different from that of nonpeated malt used for beer brewing. Lightly peated malt with lower total phenol content can be distinguished from nonpeated malt simply by the total phenol content. Seventeen volatile components were detected in the phenolic fraction of peated malt. The major components identified were phenol, *o*-cresol, guaiacol, *p*-ethylphenol, 4-methylguaiacol, 4-ethylguaiacol, and 2-phenylethanol. As minor components, furfural, 5-methylfurfural, and 5-hydroxymethylfuran were also detected. These result indicated that phenol, *o*-cresol, and guaiacol were generated from peat smoke.

Introduction

Treatment of foodstuffs with wood smoke has been widely used as one of the techniques for food preservation. In addition to this common function, flavoring effect of wood smoke has also been regarded as an important factor for the preparation of food. The aromatic components concerning smoke flavor are derived from wood constituents by thermal degradation, and the components are essentially similar to that of roast flavor. Many kinds of compounds concerning roast flavor have been identified by investigators.[2] It was found that the major components taking part in smoke flavor of wood are phenolic compounds such as guaiacol, 4-methylguaiacol, and 2,6-dimethylguaiacol.[3] Malt dried over peat fire, as raw materials for whisky brewing, has been used to provide desired smoke flavor for whisky. Studies on volatile components in whisky have been reviewed,[4] but data about flavor of peated malt have scarcely been reported in literature. Schidrowitz, *et al.*[5] indicated that phenols which were detected in a pot-stilled whisky resulted from peat smoke used for drying of malt or were produced on dried malt by heat applied in killing. Steinke, *et al.*[6] reported that the major volatile phenols such as 4-vinylphenol and 4-vinylguaiacol present in the distillate from grain alcohol fermentation were formed by the decarboxylation of *p*-coumaric acid and ferulic acid, and that the amount of these precursors increased during the cooking process. Macfarlane[7] found that total phenol content in malt increased in proportion to the amount

1) Location: *a) Iwase, Matsudo-shi, Chiba*; *b) Bunkyo-cho, Nagasaki.*

2) *a)* E. Collins, *J. Agr. Food Chem.*, **19**, 533 (1971); *b)* J. Stoffelsma, G. Sipma, D.K. Ketleus and J. Pypker, *J. Agr. Chem.*, **16**, 1000 (1968); *c)* J.P. Walradt, A.O. Pittet, T.E. Kinlin, R. Maralidhera and A. Sanderson, *J. Agr. Food Chem.*, **19**, 972 (1971); *d)* B.R. Johnson, G.R. Waller and A.L. Burlingam, *J. Agr. Food Chem.*, **19**, 1020 (1971).

3) *a)* A.E. Wasserman, *J. Food Sci.*, **31**, 1005 (1966); *b)* L.J. Bratzler, M.E. Spooner, J.E. Wetherspoon and J.A. Maxey, *J. Food Sci.*, **34**, 146 (1969).

4) *a)* L. Nykänen, E. Puputti and H. Suomalained, *J. Food Sci.*, **33**, 88 (1968); *b)* K. Nishimura and M. Masuda, *J. Food Sci.*, **36**, 819 (1971); *c)* J.H. Kahan, E.G. LaRoe and H.A. Conner, *J. Food Sci.*, **33**, 395 (1968); *d)* H.J. Wobben, R. Timmer, R. Ter Heide and P.J. de Valois, *J. Food Sci.*, **36**, 464 (1971).

5) P. Scidrowitz and F. Kaye, *J. Soc. Chem. Ind.*, **24**, 558 (1905).

6) R.D. Steinke and M.C. Paulson, *Agr. Food Chem.*, **12**, 381 (1964).

7) C. Macfarlane, *J. Inst. Brewing*, **74**, 272 (1968).

of peat used for drying, and the major volatile components in malt distillate were phenol and furfural.

The present study on the volatile components of peated malt was undertaken to determine the relation of smoke components of peat with flavor of peated malt. The results obtained from this study will be used for discrimination of peated malt from nonpeated malt.

Experimental

Materials——Malts dried over peat fire, manufactured in England, France, and Canada, were used in this experiment. These peated malts are commonly classified into three types of (1) nonpeated malts, (2) lightly peated malt (with weak smoke flavor), and (3) heavily peated malt (with strong smoke flavor). Heavily peated malt was mainly used for the detection of volatile components.

Apparatus and Conditions——Gas chromatographic analyses were carried out on a Shimadzu GC 5APF gas chromatograph equipped with a flame ionization detector. The glass column of 4 m × 3 mm was packed with 80—100 mesh Chromosorb GAW coated with 5% polyethylene glycol (PEG) 20 M. The injection port of gas chromatograph was set at 280°; column oven temperature was programmed from 80° to 250° at 5°/min; He flow rate was 60 ml/min. The mass spectrometric analyses were carried out on an LKB 9000 connected with a gas chromatograph. The coiled glass column of 3 m × 3 mm was packed with 80—100 mesh Chromosorb GAW coated with 5% PEG 20 M. Column oven temperature was programmed from 80° to 200° at 5°/min; He flow rate was 30 ml/min. The ionization voltage was 70 eV, accelarating voltage 3.5 kV, trap current was 60 μA, and separator temperature was 250°.

Preparation of Phenolic Fraction——About 500 g of malt was subjected to steam distillation under a stream of N_2 gas. After the distillate was saturated with NaCl, the distillate was extracted with ether in a separatory funnel, and 200 ml of the ether extract were extracted twice with 100 ml each of 5% $NaHCO_3$ solution in a separatory funnel. After acidification with 2N HCl, acidic component was extracted from the aqueous solution with ether, and the ether solution of acids was concentrated at 35° to approximately 0.1 ml, and used for subsequent analysis of acidic compounds.

Basic component was separated from the ether extract freed from acids by extracting with 200 ml of 2N HCl in a separatory funnel followed by conversion of the hydrochlorides into free bases with 8% aqueous NaOH and extraction with ether. The ether solution of bases was concentrated at 35° to approximately 0.1 ml, and used for subsequent analysis of basic compounds.

The ether extract freed from both acids and bases as above was extracted with a 8% NaOH solution. Phenols were obtained by acidification of the alkaline solution with 2N HCl, followed by extraction with ether. The ether solution of phenols was concentrated at 35° to approximately 0.1 ml, and used for subsequent analysis of phenolic compounds. The ether solution remaining after extraction with 8% NaOH solution contained neutral components and was used for subsequent analysis.

Quantitative Determination of Total Phenols in Malt——The method used is a modification of the method reported by Macfarlane.[7] A 10 g portion of malt was placed in a separatory funnel with 20 ml of ether saturated with 2N HCl solution, and shaken by hand for 10 min. The extraction was repeated again with 20 ml portion of ether. The ether extract was collected and transferred to a separatory funnel, 9.5 ml of 5% Na_2CO_3 solution and 0.5 ml of Folin-Denis reagent were added, and the mixture was shaken throughly during 5 min. The mixture was filtered, the filtrate was transferred to a test tube, and optical density of the filtrate was measured by a spectrophotometer at 700 nm. A calibration curve was drawn by using known concentrations of phenol solution (0—60 μg/ml) dissolved in 5% Na_2CO_3 solution.

Result and Discussion

Separation and Identification of Phenolic Components

The condensate of phenolic fraction obtained by steam distillation of peated malt possessed a cresol-like flavor and slightly sweet aroma. A typical gas chromatogram of the phenolic fraction is shown in Fig. 1. The peaks on the gas chromatogram are labeled number 1 to 31. The prominent peaks are peak 16, 18, and 23, and the relative ratio is 90% with the sum of above three peaks.

These peaks on gas chromatogram were identified by comparison with the mass spectra of authentic compounds obtained on the same instrument under similar operating conditions. Identification was further verified by comparison of retention indices of the authentic compounds with those of the unknowns.

The mass spectrum of peak 2 was compatible with that of authentic furfural and the retention index also agreed with this compound. Peak 5 was tentatively identified as 2-acetyl-

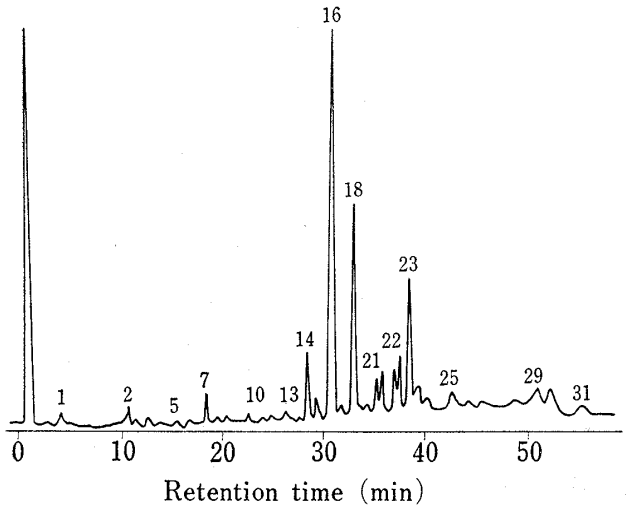

Fig. 1. Gas Chromatogram of Phenolic Fraction obtained from the Distillate of Peated Malt

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

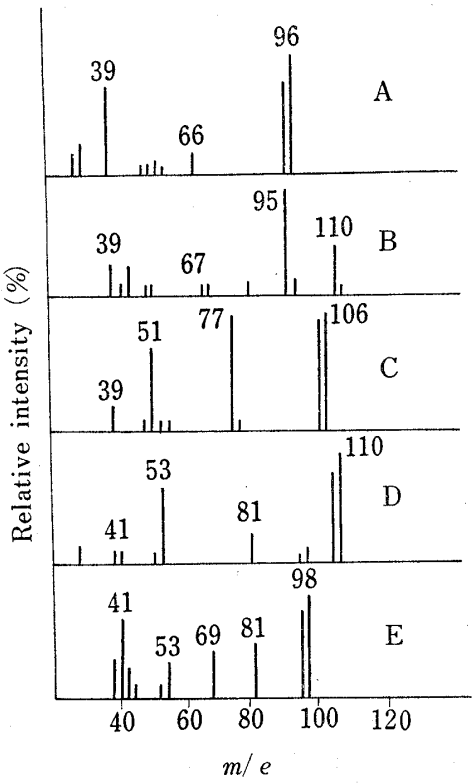

Fig. 2. Mass Spectra of Some Peaks of Phenolic Fraction

Peak numbers are the same as cited in the chromatogram of Fig. 1.
A: peak 2, B: peak 5, C: peak 6, D: peak 7, E: peak 9.

furan, and its mass spectrum was very similar to that of authentic 2-acetylfuran. Peak 6 was identified as benzaldehyde, and its mass spectrum was in close agreement with that of authentic compound. Peak 7 was identified as 5-methylfurfural, its mass spectrum and retention index being in agreement with those of authentic compound. Peak 9 was found to be 5-hydroxymethylfuran, and its mass spectrum and retention index were compatible with those of authentic compound. The mass spectra of these peaks are shown in Fig. 2.

While Mcfarlane[7] reported only the presence of furfural in the volatiles from peated malt, some furans were found in the present investigation. A number of furans have previously been isolated from several food products which underwent high-temperature processing.[8] It is most likely that furans are of carbohydrate origin and likely originate via thermal degradation. Peak 10 was identified as guaiacol, and its mass spectrum and retention index agreed closely with those of authentic compound. Peak 11 was identified as benzyl alcohol and its mass spectrum agreed with that of authentic compound.[9] Peak 12 and 14 showed similar mass spectra. In the mass spectrum of peak 12, the most intense peak was the molecular ion (M^+ 138), and other fragment ions at *m/e* 123 (95%), 95 (40%), 71 (30%), 55 (20%), and 45 (25%) were observed.

In the case of peak 14, the molecular ion (M^+ 152) was by 14 mass unit higher than that of peak 12, and fragment ion at *m/e* 137 (M^+-CH_3) was the most abundant. These mass spectra were in close agreement with those of the authentic 4-methylguaiacol (peak 12) and 4-ethylguaiacol (peak 14). Peak 19 was identified as *p*-ethylphenol, and its mass spectrum

8) T. Aczel and H.E. Lumpkin, *Anal. Chem.*, 32, 1819 (1960).
9) F.C. Dallos and K.G. Koeppl, *J. Chromatogr. Sci.*, 7, 565 (1969).

and retention index agreed with those of the authentic compound. The fragmentation pattern of peak 23 was similar to that of peak 19, but the molecular ion was by 28 mass unit higher than that of peak 19. Thus peak 23 was found to be *p*-butylphenol, and its mass spectrum was compatible with that of the authentic compound. Peak 16 was identified as phenol, its mass spectrum being in close agreement with that of the authentic compound. Peak 18 was identified as *o*-cresol. The overall spectral pattern of peak 18 shows fragmentation characteristics of alkylphenols. The mass spectrum of peak 18 was in agreement with that of the authentic *o*-cresol. Some of the phenolic compounds were also identified using their silyl derivatives by GC–mass. Gas chromatogram of silylated phenolic fraction of peated malt is shown in Fig. 3.

In general, the mass spectra of trimethylsilyl (TMS) derivatives of phenols were characterized by the presence of relatively intense molecular peaks and intense peaks resulting from the loss of methyl group from the molecular ions.[10]

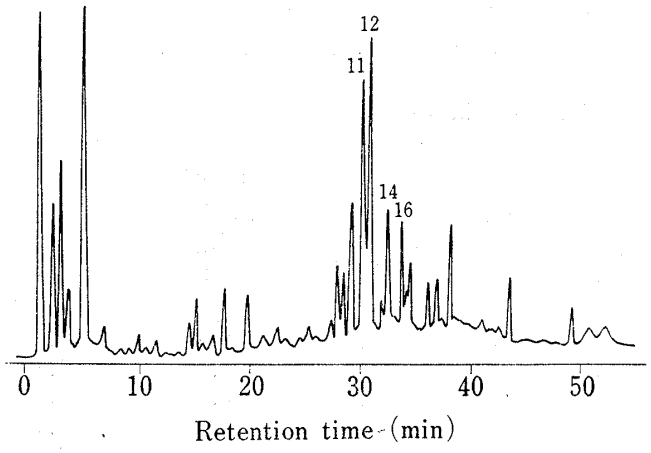

Fig. 3. Gas Chromatogram of Trimethyl silyl Derivatives of Phenolic Fraction obtained from the Distillate of Peated Malt

GC conditions are the same as cited in Fig. 1. The silylation of phenolic fraction of peated malt was as follows: 0.1 ml of N, O-bis (trimethylsilyl) acetamide was added to the same volume of phenolic fraction, and the tube sealed with a Silicon rubber septum and screw cap. The reaction mixture was shaken for 1 min and allowed to stand for 1 hr.

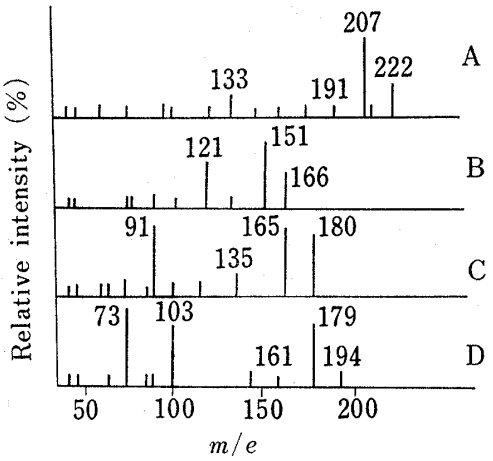

Fig. 4. Mass Spectra of Silyl Derivatives of Phenolic Fraction of Peated Malt

Peak numbers are the same as cited in chro matogram of Fig. 3.
A: peak 11, B: peak 12, C: peak 14, D: peak 16.

In the case of peak 11, the molecular ion at *m/e* 222 was clearly observed, and fragment ion at *m/e* 207 resulting from the loss of methyl group from molecular ion was the most intense peak. In the lower mass portion of the spectrum, relatively intense peaks were found at *m/e* 75, 73, 59, 45, and 43. The molecular weight of the original phenol of the derivative was easily obtained as 150 by subtraction of 72 from the molecular ion of the derivative. Peak at *m/e* 191 may be formed by the loss of OCH_3 from the molecular ion, as shown by the metastable ion at *m/e* 164.3. The mass spectrum of peak 11 was compatible with the authentic 4-vinylguaiacol trimethylsilyl ether. Peak 12 and 14 were also identified as phenol and *o*-cresol from the mass spectra of their trimethylsilyl ethers. The mass spectra of these trimethylsilyl ethers are shown in Fig. 4.

The mass spectrum of peak 16 shows molecular ion at *m/e* 194 and quite characteristic of primary alcohol trimethylsilyl ether, as evidenced by the most intense peak at *m/e* 73 and fragment ion at *m/e* 179 (M^+-CH_3). From the molecular ion of silyl ether, molecular weight of the original compound is calculated as 122. Peak 16 was identified as 2-phenylethanol, and its mass spectrum was in close agreement with that of the authentic compound.

10) M.R. Kornreich and P. Issenberg, *J. Agr. Food Chem.*, **20**, 1109 (1972).

In the phenolic fraction, the following 17 compounds were identified: Furfural, 2-acetylfuran, 5-methylfurfural, 5-hydroxymethylfuran, benzaldehyde, guaiacol, 4-methylguaiacol, 4-ethylguaiacol, phenol, *o*-cresol, *m*-cresol, *p*-cresol, *p*-ethylphenol, *p*-butylphenol, 4-vinylguaiacol, 2-phenylethanol, and benzyl alcohol.

Guaiacol, cresol, and phenol have been previously reported as volatile components in peated malt.[8] Although Steinke *et al.*[6] reported the presence of some phenolic compounds in distillate from malt alcohol fermentation, a number of phenolic compounds identified in this study have not previously been reported from the distillate of peated malt. There is a possibility that these phenolic compounds identified are making a major contribution to the smoked flavor of peated malt.

Correlation between Total Phenol Contents and Smoke Flavor

Macfarlane[7] reported that malt dried on an oil-fired kiln has a small amount of phenols, but adding peat to an oil-fired kiln further increases the phenol content. In order to evaluate the efficiency of phenolic compounds originating from peat smoke, total phenol content in several types of peated malt was analyzed. The data are shown in Table I.

Table I. Total Phenol Content in Malts[a]

Sample No.	Total phenol (ppm)			Sample No.	Total phenol (ppm)		
	Nonpeated malt	Lightly peated malt	Heavily peated malt		Nonpeated malt	Lightly peated malt	Heavily peated malt
1	1.5	3.8	8.5	6	1.6	2.1	6.1
2	1.0	2.1	7.5	7	1.8	4.8	7.8
3	1.1	4.1	11.3	8	2.0	4.5	6.9
4	0.6	4.3	8.8	9	1.8	4.1	7.1
5	0.5	4.8	15.1	10	1.1	3.9	6.1

a) malt manufactured in England

The total phenol content was below 2 ppm in nonpeated malt and 2—5 ppm in lightly peated malt. In heavily peated malt, the total phenol content is in a common ranges of 6 to 7 ppm and the value of 15 ppm at maximum. Thus, it is found that the total phenol content depends on smoke components of the fired peat. However, it is difficult to distinguish between lightly peated malt and nonpeated malt only from total phenol content. To elucidate the relation between phenolic components in peat smoke and smoked flavor of peated malt, phenolic fractions obtained from smoke solution of both English and Hokkaido peats were analyzed.

Liquid smoke solution was obtained by thermal degradation of peat in the presence of air stream at 400°, and the phenolic fraction was obtained from steam distillate of the liquid smoke solution. The gas chromatograms of the phenolic fraction are shown in Fig. 5.

Furfural, 5-methylfurfural, guaiacol, phenol, *o*-cresol, and *p*-ethylphenol were the major components in phenolic fraction of peat smoke, and both English and Hokkaido peats showed similar gas chromatogram. It is assumed that these phenolic components contribute substantially to the smoke flavor of peated malt.

As shown in Fig. 6, the intense peaks of phenol, *o*-cresol, and guaiacol are found alike in phenolic fraction of heavily peated malt imported from various countries, but *p*-ethylphenol shows a peak of a very weak intensity in the chromatogram. In the lightly peated malt, the relative ratio of phenol to *p*-ethylphenol decreases, especially in the case of peated malt from England, and the peak intensity of phenol is less than that of *p*-ethylphenol. However, in the case of nonpeated malt, extremely weak peaks of phenol, *o*-cresol, and guaiacol are observed, and conversely relatively intense peak of *p*-ethylphenol is found in the phenolic

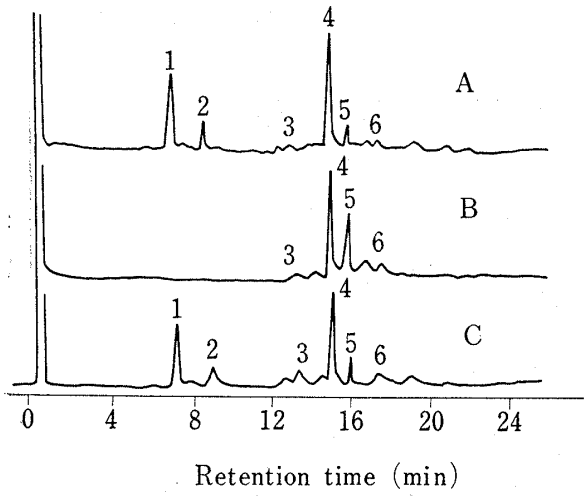

Fig. 5. Gas Chromatograms of Phenolic Fractions obtained from Liquid Smoke Solution of Peats

GC conditions are the same as cited in Fig. 1. Liquid smoke solution was obtained by thermal degradation of peat in the presence of air setram at 400°, and phenolic fraction was obtained by steam distillation of the liquid smoke solution.
A: Hokkaido peat, B: liquid smoke solution (commercial available), C: English peat
peak 1: furfural, peak 2: 5-methylfurfural, peak 3: guaiacol, peak 4: phenol, peak 5: *o*-cresol, peak 6: *p*-ethylphenol.

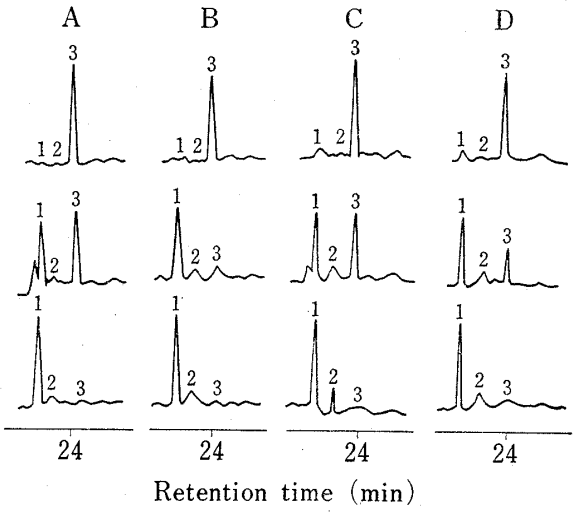

Fig. 6. Gas Chromatograms of Phenolic Fractions of Malts imported from Various Countries

GC conditions are the same as cited in Fig. 1.
(I) nonpeated malt, (II) lightly peated malt, (III) heavily peated malt
A: English malt, B: Japanese malt, C: Czechoslovakian malt, D: Canadian malt peak 1: phenol, peak 2: *o*-cresol, peak 3: *p*-ethylphenol

fraction. It is suggested from these results that phenolic compounds in peated malt depend on the smoke generated from fired peat. By comparing these phenolic components, it would be expected that the difference between peated malt and nonpeated malt can be distinguished by the ratio of phenol to *p*-ethylphenol.

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