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IDENTIFICATION OF VOLATILE COMPOUNDS IN POULTRY MANURE BY GAS CHROMATOGRAPHY–MASS SPECTROMETRY

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SUMMARY

Volatile components in poultry manure were isolated by freeze vacuum distillation and continuous extraction, and 72 compounds were identified by gas chromatography and gas chromatography–mass spectrometry. Branched alipathic alcohols, many esters, dimethyl trisulphide and alkanamides were detected for the first time. Carboxylic acids, sulphides, alkanamides, phenols and indoles were formed during rotting. Judging from the ratio of concentration to the threshold value (detectable amount of odour) of each component detected in poultry manure, the most important odorous components were butyric acid, 3-methylbutyric acid, dimethyl trisulphide, indole and skatole.

INTRODUCTION

In Japan, complaints about malodours arising from the animal industry comprise a large proportion of the total number of complaints of this type. Swine houses and poultry houses are the main sources of such odours. There have been many studies on the identification of odorous components in swine manure and in the air from swine houses. On the other hand, few studies have been reported on the identification of odorous compounds in poultry manure[1–5]. Lower carboxylic acids, ammonia, amines, indole, skatole, sulphur-containing compounds and alcohols have been detected, but satisfactory results on the volatile components have not yet been reported. The head space technique, solvent extraction or steam distillation are used most frequently for the isolation of volatile components. Freeze vacuum distillation is more effective than other isolation techniques for the analysis of volatile compounds in swine manure[6]. In this study, freeze vacuum distillation and capillary gas chromatography–mass spectrometry (GC–MS) were used for the isolation and identification of volatile compounds in poultry manure.

EXPERIMENTAL

Sampling of poultry manure

Fresh manure (9 kg) was gathered within 5 h of excretion at a large-scale

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poultry farm on May 21, 1984. The manure was mixed well and then divided into three equal parts. Each part was mixed with distilled water (600 g). The first part (sample A) was cooled rapidly to below −70°C. The second part (sample B) was kept at 27–28°C for 9 days under nearly anaerobic conditions and then frozen rapidly to below −70°C. The last part (sample C) was kept at 27–28°C for 28 days under nearly anaerobic conditions and then frozen rapidly to below −70°C.

Separation of volatile components

Volatile components in the frozen samples were isolated by freeze vacuum distillation as already described[6]. The receiver trap and the oil-trap were cooled at −100 and −195°C, respectively. The vacuum pressure was less than 10^{-4} Torr. The sample flask was kept at room temperature for 2 days and then heated at 50–60°C in a hot-air bath for the next 10 days. After melting, the material trapped in the receiver trap was subjected to continuous extraction with diethyl ether. The organic layer was separated and dried with anhydrous sodium sulphate. The distillation residue in the sample flask was mixed with diethyl ether for 1 day. The ether solution was concentrated to around 40–50 ml with a Kuderna-Danish concentrator under atmospheric pressure, then distilled under high vacuum (*ca.* 10^{-4} Torr). The distillate was added to the extract of the volatile components obtained from the first trap and then concentrated by the Kuderna-Danish concentrator under atmospheric pressure for GC and GC–MS analysis.

Analysis of volatile compounds

Identification of the volatile components was carried out by GC–MS using a cross-linked free fatty acid phase (FFAP) fused-silica capillary column (50 m × 0.25 mm I.D. × 0.25 μm). Such compounds were quantitated by GC using the same column and 1-bromononane as an internal standard. GC–MS conditions: a JEOL JMS-DX300 mass spectrometer connected with a Hewlett-Packard 5710A gas chromatograph and a JEOL JMA-3500 mass data analysis system; column temperature, 50°C for 4 min, increased to 210°C at 4°C/min; injection port temperature, 250°C; injection mode, split injection; helium flow-rate (total), 15 ml/min; enricher temperature, 250°C; ion-source temperature, 220°C; ion-source pressure, $(1–2) \cdot 10^{-6}$ Torr; electron energy, 70 eV; ionizing current, 300 μA; accelerating voltage, 3 kV; scan range, m/z 10–400; scan speed, 2.1 s per scan; repetition time, 2.5 s. GC conditions: an Hewlett-Packard 5890A gas chromatograph; column temperature, 20°C for 5 min, increased to 70°C at 2°C/min and to 210°C at 4°C/min; injection port and detector port temperatures, 250°C; injection mode, split injection; detection, flame ionization detection (FID); helium flow-rate (total), 38.5 ml/min; splitting ratio, *ca.* 80:1.

Measurement of the detectable threshold of odour

Each standard was diluted in odour-free water which was prepared by passing distilled water through an active carbon column until the odour of the diluted solution was no longer distinguishable from the odour of odour-free water.

Chemicals

Except as described below, standards were purchased from Wako Pure Chemical Industries, Tokyo Kasei Kogyo Co., Aldrich Chemical Co. and Eastman Kodak

TABLE I

AMOUNTS OF DISTILLATES AND RESIDUES IN FREEZE VACUUM DISTILLATION OF POULTRY MANURE

Sample	*Amount of distillate (g)*	*Amount of residue (g)*	*Ratio of volatiles (%)*
A	1747	1236	58.8
B	1755	1229	59.1
C	1838	1154	61.5

Co. The diacetate of 1,3-propanediol was synthesized from 1,3-propanediol and acetyl chloride. 2-Methylhexanoic acid was synthesized by the reaction of carbon dioxide and 2-hexylmagnesium chloride prepared from 2-chlorohexane and magnesium. Propanamide and butanamide were synthesized from the corresponding acyl halides and conc. ammonia aqueous solution.

RESULTS AND DISCUSSION

The amounts of distillates in the receiver trap and of residues in the sample flask are shown in Table I. The water contents in these samples were around 60%. The amount of non-volatiles decreased slightly with rotting, because of degradation

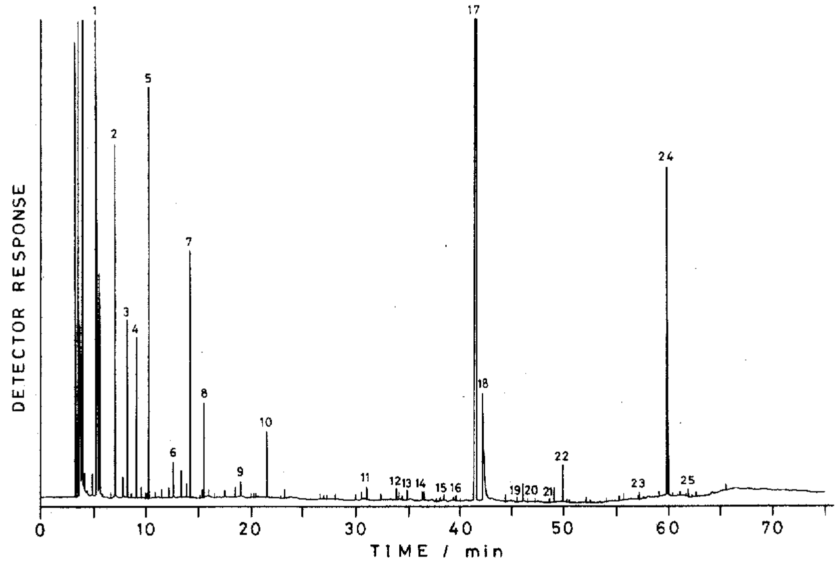

Fig. 1. Gas chromatogram of volatiles obtained from sample A of poultry manure. Peaks: 1 = ethyl formate; 2 = ethyl acetate; 3 = propyl formate; 4 = ethanol; 5 = propyl acetate; 6 = 2-methylpropyl acetate; 7 = butyl formate; 8 = propanol; 9 = propyl butyrate; 10 = butyl acetate; 11 = 3-methylbutyl acetate; 12 = butanol; 13 = pentyl acetate; 14 = pentanol; 15 = hexyl acetate; 16 = hexanol; 17 = 1-bromononane (internal standard); 18 = acetic acid; 19 = propionic acid; 20 = 2-methylpropionic acid; 21 = butyric acid; 22 = 3-methylbutyric acid; 23 = dimethyl sulphone; 24 = phenol; 25 = *p*-cresol.

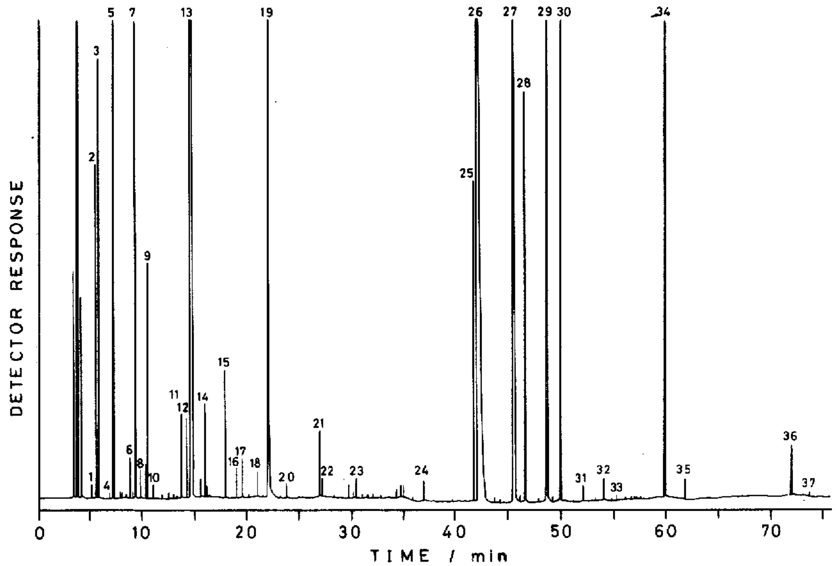

Fig. 2. Gas chromatogram of volatiles obtained from sample B of poultry manure. Peaks: 1 = propanal; 2 = acetone; 3 = ethyl formate; 4 = butanal; 5 = ethyl acetate; 6 = 2-propanol; 7 = ethanol; 8 = ethyl propionate; 9 = propyl acetate; 10 = methyl butyrate; 11 = 2-butanol; 12 = ethyl butyrate; 13 = propanol; 14 = butyl acetate; 15 = 2-methyl propanol; 16 = 2-hexanone; 17 = propyl butyrate; 18 = butyl propionate; 19 = butanol; 20 = 2-heptanone; 21 = 3-methylbutanol; 22 = butyl butyrate; 23 = pentanol; 24 = hexanol; 25 = 1-bromononane (internal standard); 26 = acetic acid; 27 = propionic acid; 28 = 2-methylpropionic acid; 29 = butyric acid; 30 = 3-methylbutyric acid; 31 = pentanoic acid; 32 = 4-methyl pentanoic acid; 33 = hexanoic acid; 34 = phenol; 35 = *p*-cresol; 36 = indole; 37 = skatole.

of non-volatile material to volatile components. Figs. 1–3 show gas chromatograms of the volatiles in samples A, B and C, respectively. Strong peaks without numbers in the figures have not yet been identified.

The results of the identifications are shown in Table II. These were made on the basis of mass spectra and retention indices. The latter, I_R, were calculated according to the method of Van den Dool and Kratz[7], using *n*-alkanes as references. Mass spectra were compared by the library search system[8]. Small amounts of many aliphatic hydrocarbons were identified by GC–MS. Part of these hydrocarbons might come from the Apiezone Grease N used for sealing purposes in the freeze vacuum distillation. This was confirmed in a blank test, where distilled water from which organic matter has first been removed by extraction with diethyl ether was used as a sample in the distillation. Therefore, aliphatic hydrocarbons are not listed in Table II. Moreover, their odours were very weak. 3-Octen-2-one and γ-hexanolactone were identified only tentatively because of the absence of standards. Benzene and some of the ethyl acetate were contaminants from the diethyl ether used as a solvent.

The alcohols content was usually higher in sample B than in sample A or C. Propanol, butanol, 3-methylbutanol and 4-methylpentanol were remarkably abundant in sample B. Most of the linear alcohols had been detected by other investigators[9], but several branched alcohols were newly identified in this study. 2-Phenylethanol, often detected in animal manure, is considered to be produced by microbial degradation of protein, although it does not play an important role on odour.

Only two aldehydes, propanal and butanal, were detected in this experiment. Their concentrations were highest in sample B and they were not detected in sample C. They might be intermediates formed during decomposition.

Four 2-alkanones were detected. These compounds are widespread among animal faeces. However, 3-methyl-2-butanone and 2,3-butadione (diacetyl) were not detected, although Burnett[2] found them in poultry manure.

Many carboxylic acids were detected and their concentrations were highest in sample C. Acetic acid, butyric acid and 3-methylbutyric acid were the most abundant. It is very interesting that no aromatic carboxylic acid was detected. In this respects poultry manure is very different from swine manure.

Many esters were found in poultry manure. It is not known whether these esters are poultry metabolites themselves or degradation products formed by microbial action after excretion. Esters do not play an important role in the malodour of poultry manure, because their odours are rather pleasant and their odour threshold values are fairly high.

Four sulphur-containing compounds were detected. Except for dimethyl sulphone, these components are well known malodorous compounds. It is not known whether highly volatile compounds such as hydrogen sulphide and methanethiol were present because the sample preparation method including vacuum distillation and solvent extraction results in loss of these two compounds, although other investiga-

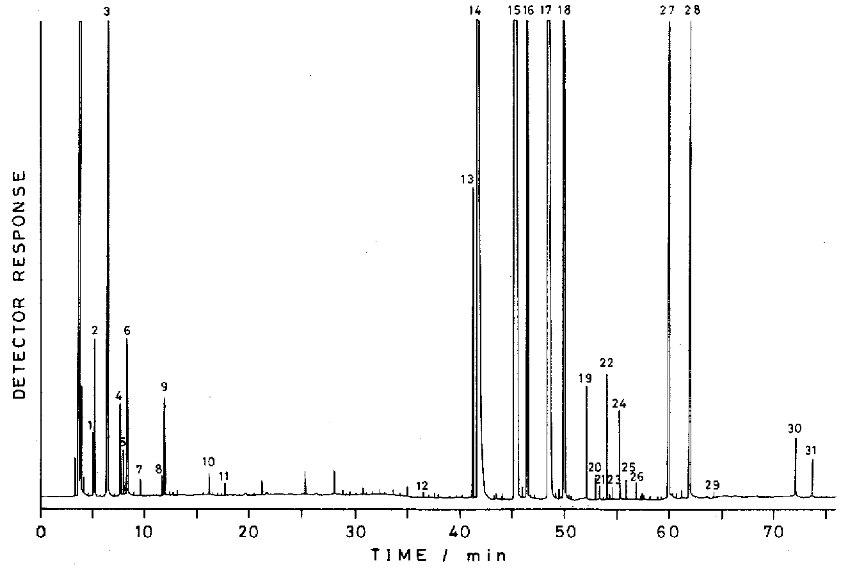

Fig. 3. Gas chromatogram of volatiles obtained from sample C of poultry manure. Peaks: 1 = acetone; 2 = ethyl formate; 3 = ethyl acetate; 4 = 2-propanol; 5 = benzene; 6 = ethanol; 7 = 2-butyl acetate; 8 = 2-butanol; 9 = ethyl butyrate; 10 = 2-hexanone; 11 = 2-pentanol; 12 = dimethyl trisulphide; 13 = 1-bromononane (internal standard); 14 = acetic acid; 15 = propionic acid; 16 = 2-methylpropionic acid; 17 = butyric acid; 18 = 3-methylbutyric acid; 19 = pentanoic acid; 20 = 2-methylpentanoic acid; 21 = acetamide; 22 = 4-methylpentanoic acid; 23 = propanamide; 24 = hexanoic acid; 25 = 2-methylhexanoic acid; 26 = butanamide; 27 = phenol; 28 = *p*-cresol; 29 = *p*-ethylphenol; 30 = indole; 31 = skatole.

TABLE II

CONCENTRATIONS OF VOLATILE COMPONENTS AND THEIR GC RETENTION INDICES

ND = not determined.

Compound	*Retention index*	*Concentration (mg/kg)*		
		A	*B*	*C*
Ethanol	935	3.81	48.0	22.6
1-Methylethanol	924	ND	8.78	3.30
Propanol	1031	3.33	118	ND
2-Butanol	1015	0.150	4.63	6.58
2-Methylpropanol	1073	0.105	2.96	1.22
Butanol	1136	0.391	61.3	0.480
2-Pentanol	1112	ND	ND	1.04
3-Methyl-2-butanol	1118	ND	ND	0.139
2-Methylbutanol	1191	ND	0.460	1.58
3-Methylbutanol	1197	0.398	4.01	ND
Pentanol	1240	0.184	0.768	0.370
3-Methyl-3-butenol	1237	ND	0.112	0.322
4-Methylpentanol	1310	0.019	0.369	ND
Hexanol	1346	0.216	0.527	ND
2-Phenylethanol	1926	ND	0.014	ND
Propanal	784	0.059	0.299	ND
Butanal	862	0.008	0.207	ND
Acetone	808	0.636	5.88	41.5
2-Butanone	882	ND	0.151	0.247
2-Hexanone	1083	ND	0.695	0.830
2-Heptanone	1154	0.046	0.235	ND
Acetic acid	1462	9.17	235	464
Propionic acid	1551	1.11	49.5	241
2-Methylpropionic acid	1578	0.430	21.1	68.5
Butyric acid	1640	0.745	108	509
3-Methylbutyric acid	1681	0.896	35.8	484
Pentanoic acid	1752	ND	0.906	4.40
2-Methylpentanoic acid	1775	ND	ND	0.306
4-Methylpentanoic acid	1817	ND	1.28	3.76
Hexanoic acid	1860	0.012	0.315	2.86
2-Methylhexanoic acid	1864	ND	ND	0.209
Octanoic acid	2050	ND	ND	0.091
Ethyl formate	820	29.6	10.9	14.9
Propyl formate	892	0.449	0.168	ND
Butyl formate	1010	0.447	0.041	ND
Ethyl acetate	868	10.8	23.0	61.2
1-Methylethyl acetate	915	ND	ND	0.166
Propyl acetate	960	0.670	3.54	ND
2-Butyl acetate	974	0.055	ND	0.617
2-Methylpropyl acetate	1004	0.050	0.168	ND
Butyl acetate	1065	0.074	1.39	ND
3-Methylbutyl acetate	1128	0.015	ND	ND
Pentyl acetate	1162	0.011	ND	ND
Ethyl propionate	945	0.031	1.03	0.767
2-Methylpropyl propionate	1069	ND	0.099	ND
Butyl propionate	1129	ND	0.642	ND
Ethyl 2-methylpropionate	952	0.009	0.054	0.077
Methyl butyrate	971	ND	0.272	0.287

TABLE II (*continued*)

Compound	*Retention index*	*Concentration (mg/kg)*		
		A	*B*	*C*
Ethyl butyrate	1021	0.010	1.35	2.69
Propyl butyrate	1098	0.022	1.14	ND
Butyl butyrate	1210	ND	0.817	ND
3-Methylbutyl butyrate	1257	ND	ND	0.287
Pentyl butyrate	1305	ND	0.059	0.239
Ethyl 3-methylbutyrate	1057	ND	0.085	ND
Propyl 3-methylbutyrate	1145	0.206	ND	ND
Diacetate of 1,3-propanediol	1655	ND	0.038	ND
Dimethyl sulphide	733	ND	ND	0.224
Dimethyl disulphide	1053	ND	0.437	0.625
Dimethyl trisulphide	1372	ND	ND	0.758
Dimethyl sulphone	1932	0.052	0.098	0.094
Acetamide	1788	ND	0.070	0.402
Propanamide	1822	ND	0.018	0.305
Butanamide	1901	ND	ND	0.690
Phenol	2028	1.86	28.0	68.1
p-Cresol	2105	0.059	0.796	34.2
p-Ethylphenol	2198	ND	ND	0.063
Indole	2446	ND	5.08	10.4
Skatole	2489	ND	0.131	1.05
2-Methylpyridine	1190	0.247	ND	0.667

tors[3,4] described the detection of a few sulphur-containing compounds. This is the first report of the detection of dimethyl trisulphide.

Alkanamides were detected in poultry manure for the first time. They do not play a significant role in malodour since their odour is very weak.

Three phenol compounds were found in poultry manure. Phenol was the most abundant in every sample. Since phenol compounds are excreted as non-toxic conjugates such as glucuronides, they are probably liberated again by microbial or enzymatic action after excretion.

Three nitrogen-containing compounds were identified. Indole and skatole are very widespread components in animal faeces. It is noteworthy that neither indole nor skatole was contained in sample A. Both compounds were formed during rotting. Detection of 2-methylpyridine was rare.

The compounds detected here can be divided into three types. The first type is a group of compounds which are present in fresh manure and rapidly decrease with rotting. Esters of formic acid are typical. The second type comprises compounds whose concentrations become higher with rotting. 2-Alkanone, carboxylic acids, alkanamides, sulphur-containing compounds, phenols and indoles belong to this type. The last type contains compounds whose concentrations increase in the early stage of rotting and decrease after long-term rotting, *e.g.*, alcohols and aldehydes.

The detectable thresholds of odour are shown in Table III where only compounds whose concentrations in poultry manure exceeded the threshold values among 65 odorous components measured are listed. It is very useful to compare the concentration of each component with its threshold of odour in order to evaluate the

TABLE III

DETECTABLE THRESHOLD OF ODOUR OF SOME IMPORTANT COMPONENTS IN WATER

These compounds were found in higher concentration than the odour threshold values in poultry manure.

Compound	*Threshold (mg/kg)*
2-Heptanone	0.20
Butyric acid	33
3-Methylbutyric acid	22
Hexanoic acid	2.0
Propyl butyrate	0.83
Dimethyl disulphide	0.63
Dimethyl trisulphide	0.083
p-Cresol	6.7
Indole	0.25
Skatole	0.067

important component of the malodour, although it is not certain at the present time that the odour strength of poultry manure is related directly to that of an aqueous solution of each component. As a result, butyric acid, 3-methylbutyric acid, dimethyl trisulphide, indole and skatole were very important components from the viewpoint of the odour concentration, which is defined as the ratio of the concentration to the odour threshold value. Lower carboxylic acids, indole and skatole are widespread compounds in animal manure. Several investigators[10–13] have reported that dimethyl trisulphide plays a significant role in malodour. In conclusion, the main malodorous components in poultry manure were butyric acid, 3-methylbutyric acid, dimethyl trisulphide, indole and skatole.

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