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ISOLATION AND ANALYSIS OF ODOROUS COMPONENTS IN SWINE MANURE

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SUMMARY

Systematic procedures are described for the isolation and extraction of odorous components in swine faeces, urine and rotten mixtures of swine faeces and urine. Samples were frozen and subjected to vacuum distillation in the frozen state. The distillate was continuously extracted with diethyl ether. The residue was extracted with diethyl ether and the extract was subjected to vacuum distillation. The former extract and the latter distillate were combined and concentrated. Recovery by these procedures was considered. Odorous compounds isolated were analyzed by gas chromatography and gas chromatography–mass spectrometry.

INTRODUCTION

An offensive odour is an important environmental factor, particularly in residential areas. The problem has been a significant one in Japan for some ten years, but few investigations have been performed. Because an offensive odour is not really harmful, far more effort has been focused on other forms of pollutions, such as photochemical smog and toxic compounds. Usually the atmosphere is analysed to obtain some information on odour, but this method is not sufficient for the characterization of odourous compounds because the odour threshold of offensive compounds is often very low. Moreover, other compounds from automobile exhausts, effluent gases from some factories, etc., are mixed with the original odorous compounds. If malodorous compounds are to be characterized, their source must first be identified.

Because odorous compounds are always volatile, it is necessary to separate volatile from non-volatile compounds in samples when odorous components in liquid or solid samples are investigated. The separation techniques summarized in Table I are often used in an investigation of flavours and aromas[1–3]. Separation of volatile from non-volatile substances is not necessary for gaseous samples. The head-space gas technique and the gas-stripping technique do not reflect the correct distribution of volatile compounds in liquid samples, because of insufficient equilibrium and of unknown coefficients of air–liquid distribution in complex systems. But the gas-strip-

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TABLE I

ISOLATION METHODS FOR VOLATILE COMPOUNDS

Sample form	*Isolation method*	
Gas	Direct analysis	
	Cold trapping	
	Adsorption by porous adsorbent	
	Absorption by cold solvent	
	Chemical derivatization	
Liquid	Head-space gas	
	Gas stripping	
	Distillation under atmospheric pressure	Gas stripping
	Distillation under reduced pressure	Continuous extraction
	Flash distillation under reduced pressure	Adsorption by resins
	Steam distillation	
	Vacuum distillation under frozen state	
Solid	Head-space gas	
	Vacuum distillation (sublimation)	
	Solvent extraction	Vacuum distillation

ping method or the gas purging-and-trapping method is satisfactory for compounds that are very volatile and fairly insoluble in water[4]. Distillation under reduced pressure and flash distillation under reduced pressure are not useful for fluids such as urine, because foaming occurs, although the quality of the odour does not change during distillation. When an aqueous sample is heat-stable and does not foam, a Nickerson–Likens apparatus[5], which is a combination of circulated steam distillation and continuous extraction, is recommended because it prevents contamination by steam or solvent. This apparatus was applied to an environmental sample and showed good results[6]. In most cases, an offensive odour is readily changed by heating, and environmental liquid samples foam during distillation, therefore these separation methods are rarely applicable. Vacuum distillation in the frozen state is most useful for such samples.

This paper describes the isolation and analysis of odorous compounds in swine manure.

EXPERIMENTAL

Samples and procedures of isolation and extraction of volatile components

As an example of liquid and solid samples, swine urine and faeces were sampled within 12 h after excretion in the National Institute of Animal Industry. Raw faeces (1000 g, Sample A) were frozen at −20°C immediately after sampling. Urine (1000 ml, Sample B) was frozen in the same way. A mixture (Sample C: hereafter called the "rotten mixture") of urine (860 ml) and faeces (228 g) was kept at room temperature (15–19°C) for a week, and then frozen at −20°C. The frozen samples were further cooled at −80°C for more than 10 h and then vacuum-distilled while still frozen (Fig. 1). The vacuum pressure was kept at *ca.* 10^{-4} torr. A receiver trap and an oil trap were cooled at −100 and −195°C, respectively. The sample flask was kept at room temperature for 1 day, heated at *ca.* 70°C in a hot-air bath for the next

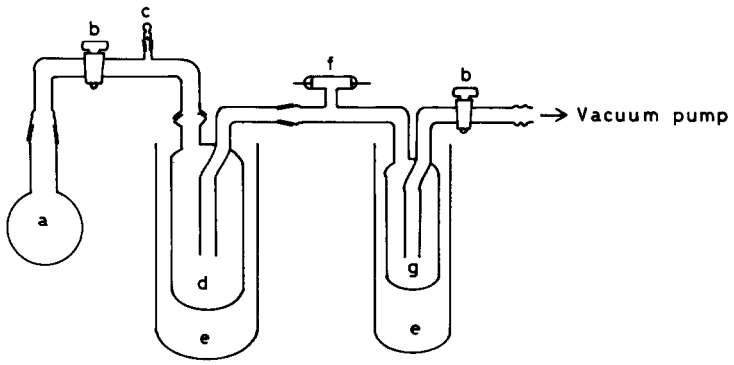

Fig. 1. Apparatus for vacuum distillation in the frozen state. a = Sample flask; b = two-way valve; c = leak valve; d = receiver trap; e = Dewar bottle; f = Geissler tube; g = oil trap.

1 or 2 days and then heated to *ca.* 100°C by a mantle heater until no more water or volatiles were collected in the receiver trap. The period for final heating process was between 3 to 7 days and dependent on amount of non-volatile material present. The distillate was heated slowly until it melted. The water-containing volatile compounds were subjected to continuous extraction for 24 h (Fig. 2) with diethyl ether, which was redistilled through a Widmer column (45 cm length) immediately before use. The organic layer was separated and dried with anhydrous sodium sulphate. The residue

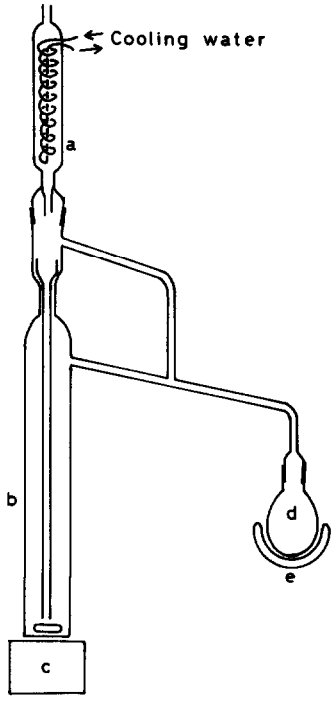

Fig. 2. Apparatus for continuous extraction. a = Condenser; b = sample vessel for aqueous solution; c = magnetic stirrer; d = solvent flask; e = mantle heater.

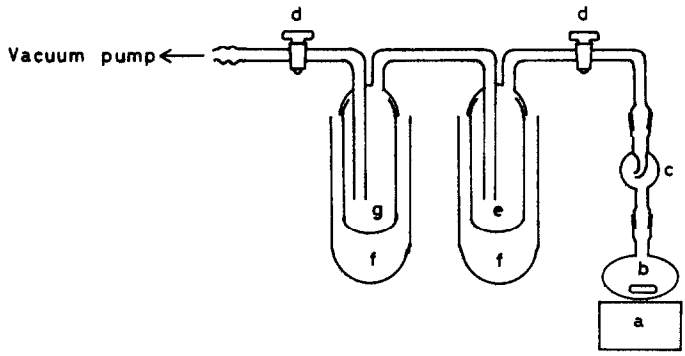

Fig. 3. Apparatus for vacuum distillation of extracts from distillation residue. a = Magnetic stirrer; b = sample flask; c = bulb; d = three-way valve; e = receiver trap; f = Dewar bottle; g = oil trap.

in the sample flask was mixed with diethyl ether, which was similarly redistilled, and the remaining compounds were extracted for 24 h. The extracted solution was concentrated to *ca.* 40–50 ml after filtration. The concentrated solution was vacuum-distilled (Fig. 3). The vacuum pressure was *ca.* 10^{-4} torr and both traps were chilled by liquid nitrogen. The distillate was combined with the organic layer described above. The combined solution was concentrated to a few millilitres under atmospheric pressure using a Snyder column (20 cm length).

Recovery of odorous compounds

The following compounds were dissolved in 1 l of distilled water and then frozen at −80°C overnight: 1-methylethanol (3.9 mg); dimethyl disulphide (42.4 mg); 2,3-dimethyl-2-butanol (4.0 mg); 3-pentanol (4.1 mg); 2-hexanol (4.1 mg); 3-hydroxy-2-butanone (4.2 mg); 2,5-dimethylpyrazine (4.9 mg); acetic acid (52.2 mg); benzaldehyde (5.1 mg); propanoic acid (48.2 mg); 2-methylpropanoic acid (47.4 mg); butanoic acid (48.0 mg); 3-methylbutanoic acid (45.5 mg); pentanoic acid (9.5 mg); 4-methylpentanoic acid (0.9 mg); 2-methoxyphenol (5.6 mg); phenylmethanol (5.1 mg); 2-phenylethanol (5.1 mg); phenol (9.0 mg); 4-methylphenol (12.4 mg); 4-ethylphenol (11.2 mg); benzoic acid (4.0 mg); 3-methylindole (4.0 mg); and phenylacetic acid (4.2 mg). Vacuum distillation was carried out as described above, although it was not necessary to wash the sample flask with diethyl ether after distillation.

Analysis of volatile components

Quantification and identification of volatile components were carried out by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS). Quantification was carried out using peak heights in gas chromatograms without an internal standard. Calibration curves of some compounds are shown in Fig. 4. GC conditions for the packed column were as follows: glass column, 5% Thermon-3000 (Chromosorb W, 80–100 mesh, 3 m × 3 mm I.D.); column temperature, 50°C for 2 min, followed by an increase to 210°C at 4°C/min; injection port temperature, 250°C; carrier gas (nitrogen) flow-rate, 50 ml/min. GC conditions for the capillary column were as follows: column, Carbowax 20M fused-silica open tubular column (50 m × 0.2 mm I.D.); column temperature, 50°C for 5 min, followed by an increase to 200°C at 4°C/min; injection port temperature, 250°C; carrier gas (nitrogen) flow-rate,

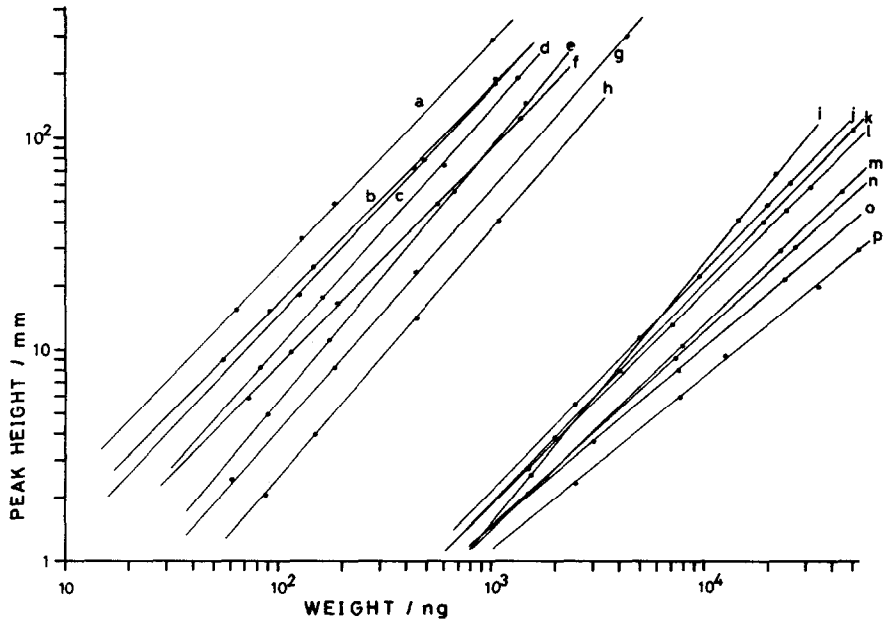

Fig. 4. Calibration curves of some compounds. The range in the gas chromatograph was 10^3 (10^7 ohm). (a) 2,3-Dimethyl-2-butanol (attenuation, 1); (b) 3-pentanol (attenuation, 1); (c) 3-methylbutanol (attenuation, 1); (d) 2-phenylethanol (attenuation, 1); (e) 2-methoxyphenol (attenuation, 1); (f) dimethyl disulphide (attenuation, 1); (g) dimethylsulphone (attenuation, 1); (h) 3-hydroxy-2-butanone (attenuation, 1); (i) phenylacetic acid (attenuation, 4); (j) skatole (attenuation, 8); (k) phenol, 4-methylphenol and 4-ethylphenol (attenuation, 8); (l) indole (attenuation, 8); (m) hexanoic acid (attenuation, 8); (n) pentanoic acid (attenuation, 8); (o) 2-methylpropanoic acid (attenuation, 8); (p) propanoic acid (attenuation, 8).

20 ml/min; split ratio, *ca.* 50:1. GC conditions in GC–MS were the same as in GC except that helium was used as carrier gas. MS conditions were as follows: apparatus, Jeol JMS-DX 300 mass spectrometer connected with Hewlett-Packard HP5710A gas chromatograph and Jeol JMA 3500 mass data analysis system; ionizing current, 300 μA; electron energy, 70 eV; accelerating voltage, 3 kV; ion-source pressure, 1–2 × 10^{-6} torr; ion-source temperature, 220°C; separator temperature, 250°C; scan range, m/z 10 to 400; scan speed, 1.3 sec/scan; repetition time, 2 sec. A library search system[7,8], was sometimes used for identification of mass spectra.

RESULTS AND DISCUSSION

During vacuum distillation, the sample remains frozen and there is hardly any foaming. Water vapour and volatile compounds are sublimated from the surface of the sample. It should be noted that the vacuum pressure is kept at *ca.* 10^{-4} torr, a receiver trap is cooled at *ca.* −100°C or less and a glass pipe, which connects the sample flask with the receiver trap, is as short and thick as possible in order to prevent adsorption of volatile compounds onto the wall of the glass pipe, which occurs with fatty acids and phenols. Urine samples must be cooled sufficiently before distillation (*ca.* −80°C). Unless this operation is performed, vigorous foaming occurs immediately after distillation starts. The sample flask must be kept at room temperature for

several hours, then carefully heated in order to shorten the distillation period. In this study, an air bath and a mantle heater with temperature control devices were used for heating. The temperature of the sample flask must be carefully controlled, because too high a temperature causes the sample to decompose. The total period of the distillation varies with the nature of sample: the larger the amount of non-volatile compounds a sample contains, the longer is needed for the distillation. To determine the end of a distillation, the best way is to examine whether a sample flask cools to below room temperature by loss of evaporation heat after heating stops. Odorous compounds in rotten blue-green algae were separated successfully by this distillation method[9]. A continuous extractor is very useful for the extraction of volatile compounds from aqueous solution, one reason being that only a small amount of solvent is needed so contamination from the solvent is minimized. A second reason is that complete extraction is possible even if some compounds are partitioned more into water than into the organic solvent. Because very volatile compounds are easily lost during extract concentration and masked during solvent elution from a gas chromatograph, the choice of solvent for the extraction is very important. Among organic solvents that can be handled safely at room temperature and have a low boiling point, diethyl ether and dichloromethane were considered. Dichloromethane sometimes showed contaminants such as trichloroethene, tetrachloroethene and chlorinated benzenes even after redistillation, though this solvent is recommended in the literature[10]. On the other hand, diethyl ether did not contain any contaminant after distillation, and was therefore chosen as the extraction solvent. When the extracted solution is concentrated, evaporation of solvent by a rotary evaporator under reduced pressure or by blowing inert gas should be avoided, otherwise loss of more volatile compounds occurs. Removal of diethyl ether by distillation under atmospheric pres-

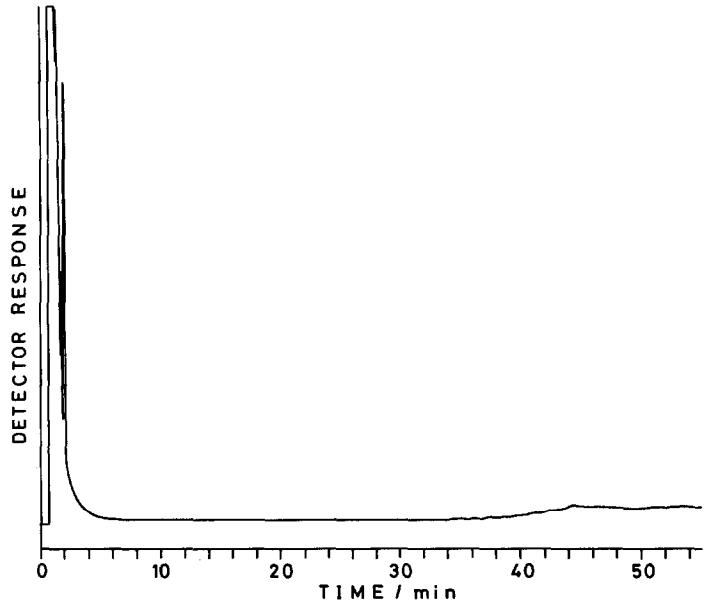

Fig. 5. Gas chromatogram of solvent (diethyl ether) blank.

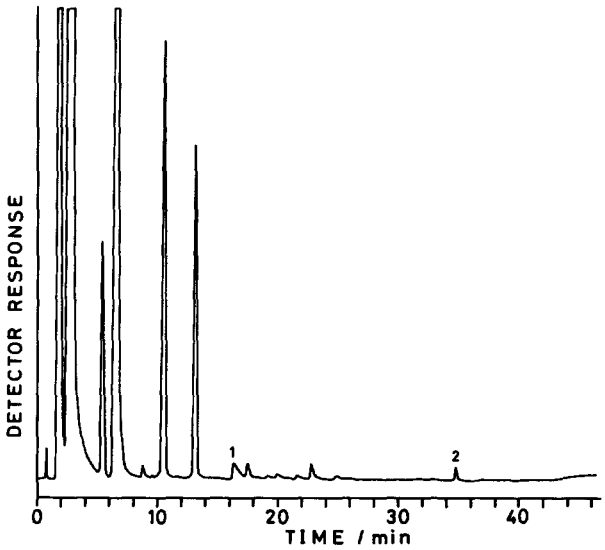

Fig. 6. Gas chromatogram of the second extract from distillation residue. 1 = Acetic acid; 2 = 4-methylphenol; all other peaks were due to contaminants in acetone.

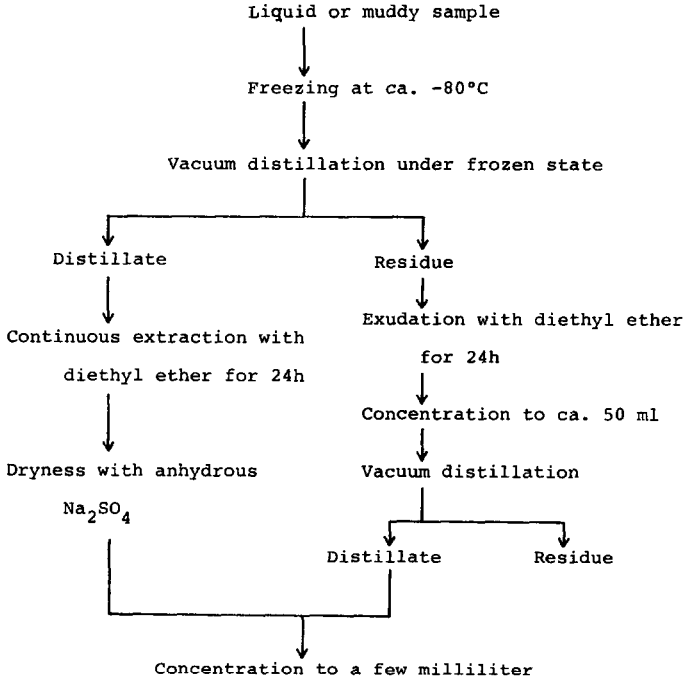

Fig. 7. Overall procedure for isolation of volatile compounds in liquid or muddy samples.

sure through a Snyder column or any other suitable column is preferable. A blank of diethyl ether is shown in Fig. 5, when 300 ml of diethyl ether were concentrated to 1 ml by the method described here. The result was very satisfactory.

In order to check the isolation efficiency of odorous compounds from a sample, sniffing the residue by human nose is both convenient and sensitive. But it should be noted that the residue is not necessarily completely odourless. In general, some components remain in the residue when the sample contains significant amounts of non-volatile substances. Solvent extraction of the remaining compounds in the distillation residue is then necessary. Not only volatile compounds but also non-volatile materials are extracted by this operation. Therefore, it is necessary again to separate volatile compounds from non-volatile materials by using the apparatus shown in Fig. 3. To make sure that the remaining volatile compounds were mostly extracted by this operation, further extraction from the residue after the first extraction was carried out with acetone (Fig. 6, where the recorder gain was increased eight times). Only trace amounts of acetic acid and 4-methylphenol were detected. Other peaks in Fig. 6 were due to contaminants in acetone.

The overall procedure for isolation of volatile compounds in liquid or muddy samples is summarized in Fig. 7. In cases where rotten blue-green algae were used as a sample, volatile compounds were isolated by vacuum distillation only in the frozen state[9], because the amount of the residue was very small. On the other hand, volatile compounds remained in the residue from vacuum distillation of samples of swine manure, when the amount of the residue was larger. In all cases, fatty acids and phenols were detected in the extracts from the residue. These compounds may attach to polar functional groups in non-volatile substances by means of hydrogen bonds. Because fatty acids and phenols have extremely low odour thresholds[11], they contribute greatly to bad smells even if their vapour pressure decreases by formation of hydrogen bonds.

Recoveries of some compounds by this method, except for an extraction from the distillation residue, were investigated by using aqueous solution of the authentic compounds (Table II). The low recovery of dimethyl disulphide may be due to its

TABLE II

RECOVERY OF ODOROUS COMPOUNDS FROM AQUEOUS SOLUTION BY VACUUM DISTILLATION

Compound	*Recovery (%)*	*Compound*	*Recovery (%)*
1-Methylethanol	100	3-Methylbutanoic acid	95
Dimethyl disulphide	30	Pentanoic acid	91
2,3-Dimethyl-2-butanol	92	4-Methylpentanoic acid	94
3-Pentanol	100	2-Methoxyphenol	87
2-Hexanol	90	Phenylmethanol	113
3-Hydroxy-2-butanone	50	2-Phenylethanol	98
2,5-Dimethylpyrazine	88	Phenol	96
Acetic acid	90	4-Methylphenol	96
Benzaldehyde	112	4-Ethylphenol	89
Propanoic acid	95	Benzoic acid	66
2-Methylpropanoic acid	91	3-Methylindole	85
Butanoic acid	109	Phenylacetic acid	64

high volatility, and might be improved by using liquid nitrogen as a coolant in the receiver trap. Poor recoveries of 3-hydroxy-2-butanone, benzoic acid and phenylacetic acid remain unexplained, although they may react with other compounds or have lower volatility. For environmental samples that contain large amounts of non-volatile material and minerals, estimation of recovery by spiking with authentic compounds was essentially impossible for the same reason as described in the case of soil and sediment[12]. In preliminary experiments (not described here) recoveries of spiked fatty acids, phenols and carbonyl compounds showed variation. These compounds

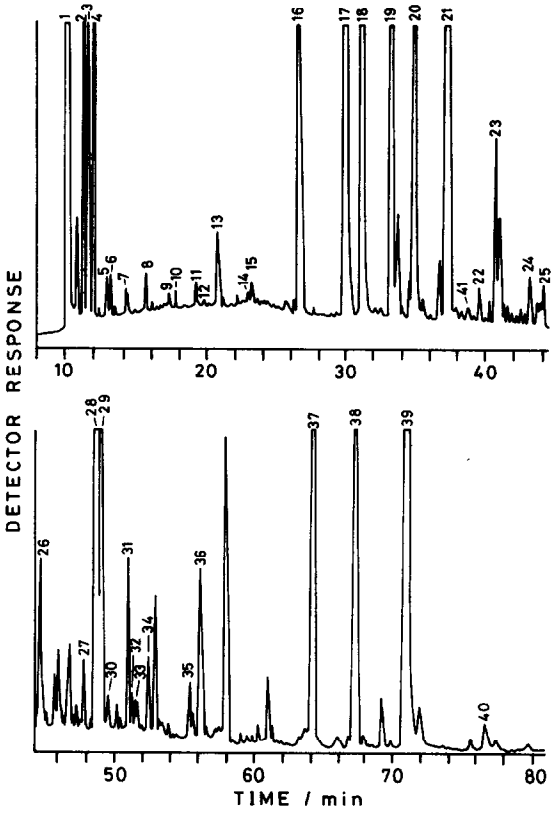

Fig. 8. Gas chromatogram of volatile components in swine faeces (Sample A). Column, Carbowax 20M fused-silica capillary column (50 m × 0.2 mm I.D.). Peaks: 1 = diethyl ether; 2 = ethyl acetate; 3 = ethanol; 4 = benzene; 5 = 1-methylethanol; 6 = propanol; 7 = 2-methylpropanol; 8 = butanol; 9 = 2-heptanone; 10 = 3-methylbutanol; 11 = methyl 2-oxopropanoate (tentatively assigned); 12 = pentanol; 13 = 3-hydroxy-2-butanone; 14 = *n*-tridecane; 15 = 3-methylpentanol (tentatively assigned); 16 = acetic acid; 17 = propanoic acid; 18 = 2-methyl-propanoic acid; 19 = butanoic acid; 20 = 3-methylbutanoic acid; 21 = pentanoic acid; 22 = hexanoic acid; 23 = *n*-octadecane; 24 = hexahydrofarnesol (tentatively assigned); 25 = *n*-nonadecane; 26 = phenol; 27 = 2-pentadecanone (tentatively assigned); 28 = 4-methylphenol; 29 = 3-methylphenol; 30 = hexadecanone (tentatively assigned); 31 = farnesyl acetate (tentatively assigned); 32 = 2-hexadecanone (tentatively assigned); 33 = 4-ethylphenol; 34 = *n*-heneicosane; 35 = ethyl hexadecanoate; 36 = tetradecanol (tentatively assigned); 37 = hexadecanol (tentatively assigned); 38 = indole; 39 = skatole; 40 = dodecanoic acid; 41 = 4-methylpentanoic acid. Unnumbered peaks were not identified.

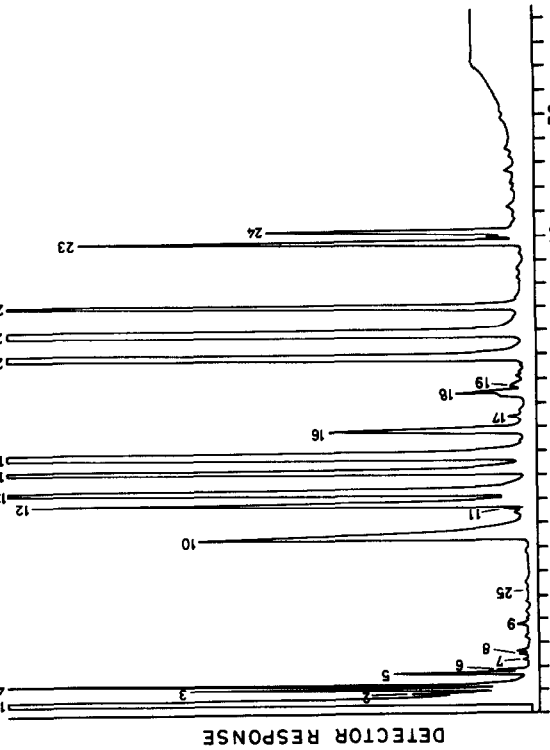

Fig. 9. Gas chromatogram of volatile components in swine urine (Sample B). Glass column, 5% Thermon-3000 on Chromosorb W (3 m × 3 mm I.D.). Peaks: 1 = diethyl ether; 2 = ethyl acetate; 3 = ethanol; 4 = benzene; 5 = propanol; 6 = 3-hydroxy-2-butanone; 7 = acetic acid; 8 = propanoic acid; 9 = 2-methylpropanoic acid; 10 = butanoic acid; 11 = 3-methyl-butanoic acid; 12 = pentanoic acid; 13 = 2-methyl-2-butenoic acid (tentatively assigned); 14 = 2-methoxyphenol; 15 = dimethylsulphone; 16 = 3-hydroxy-2-methyl-4*H*-pyran-4-one; 17 = phenol; 18 = 4-methylphenol and 3-methylphenol; 19 = 4-ethylphenol; 20 = 4-methoxyphenol; 21 = benzoic acid; 22 = phenylacetic acid; 23 = 3-phenylpropanoic acid. Unnumbered peaks were not identified.

Fig. 10. Gas chromatogram of volatile components in rotten mixture of swine faeces and urine (Sample C). Glass column, 5% Thermon-3000 on Chromosorb W (3 m × 3 mm I.D.). Peaks: 1 = diethyl ether; 2 = ethyl acetate; 3 = ethanol; 4 = benzene; 5 = 1-methylethanol; 6 = toluene; 7 = 5-hexen-2-ol (tentatively assigned); 8 = 2,3-dimethyl-2-butanol; 9 = 3-methylbutanol; 10 = acetic acid; 11 = benzaldehyde; 12 = propanoic acid; 13 = 2-methylpropanoic acid; 14 = butanoic acid; 15 = 3-methylbutanoic acid; 16 = pentanoic : acid; 17 = *n*-octadecane; 18 = dimethylsulphone; 19 = 2-phenylethanol; 20 = phenol; 21 = 4-methylphenol and 3-methylphenol; 22 = 4-ethylphenol; 23 = indole; 24 = skatole; 25 = 3-octanone (tentatively assigned). Unnumbered peaks were not identified.

may react with alkaline substances, amino compounds, etc., which are contained in the samples.

The amounts of the distillate and the residue in Samples A, B and C were 710 ml and 282 g, 950 ml and 53 g, and 965 ml and 123 g, respectively. The water content (72%) of faeces agreed with a value[13] reported previously. Gas chromatograms of volatile extracts from Samples A, B and C are shown in Figs. 8, 9 and 10. Analytical results are shown in Table III. Odorous compound contents are greatly affected by

TABLE III

ANALYTICAL RESULTS OF ODOROUS COMPONENTS IN SAMPLES A, B AND C

Compound	*Concentration (ppm)*		
	Sample A	*Sample B*	*Sample C*
1-Methylethanol	0.076	n.d.*	1.04
Propanol	0.102	0.127	n.d.
2-Methylpropanol	0.127	n.d.	n.d.
Butanol	0.127	n.d.	n.d.
2-Heptanone	0.090	n.d.	n.d.
2,3-Dimethyl-2-butanol	n.d.	n.d.	0.068
3-Methylbutanol	0.076	n.d.	0.085
Pentanol	0.014	n.d.	n.d.
3-Hydroxy-2-butanone	0.745	1.06	n.d.
3-Octanone**	n.d.	n.d.	0.018
3-Methylpentanol**	0.073	n.d.	n.d.
Acetic acid	11.8	8.13	17.9
Benzaldehyde	n.d.	n.d.	0.127
Propanoic acid	31.6	0.223	11.7
2-Methylpropanoic acid	10.3	0.861	12.6
Butanoic acid	33.9	0.493	18.0
3-Methylbutanoic acid	36.1	2.77	37.5
Pentanoic acid	26.6	0.521	2.50
2-Methyl-2-butenoic acid**	n.d.	1.20	n.d.
4-Methylpentanoic acid	0.384	n.d.	n.d.
Hexanoic acid	1.21	n.d.	n.d.
2-Methoxyphenol	n.d.	4.18	n.d.
Dimethylsulphone***	n.d.	3.47	1.42
2-Phenylethanol	0.237	n.d.	0.120
3-Hydroxy-2-methyl-4*H*-pyran-4-one	n.d.	0.708	n.d.
Phenol	0.497	10.4	45.4
2-Pentadecanone**	0.254	n.d.	n.d.
4-Methylphenol	28.5	111	73.0
2-Hexadecanone**	0.141	n.d.	n.d.
4-Ethylphenol	0.480	12.3	3.75
4-Methoxyphenol	n.d.	0.702	n.d.
Indole	3.39	n.d.	3.98
Skatole	8.35	n.d.	1.87
Benzoic acid	n.d.	101	n.d.
Phenylacetic acid	n.d.	18.7	n.d.
3-Phenylpropanoic acid	n.d.	6.17	n.d.

* Not detected.

** These compounds were tentatively assigned by GC–MS.

*** This compound is odourless.

feed contents. It was also suggested that concentrations of odorous compounds in swine faeces changed with growth of the pigs[13]. Organoleptic tests on swine manure have shown that vacuum distillation is the best method for isolating similar odorous components to original sample[14,15]. The number of odorous components in faeces (Sample A) was higher than in urine (Sample B). Fatty acids were more abundant in faeces than in urine, probably because production of fatty acids may be due to microbial action[16]. On the other hand, phenols and aromatic carboxylic acids were more abundant in urine than in faeces, because these compounds may be produced by metabolism or enzymes[17,18]. The main components in the odour of sample C were fatty acids, phenols and indoles. Changes in the fatty acids content of stored slurry were investigated by some researchers[19,20]. It is interesting that aromatic carboxylic acids are not present in the rotten mixture of swine faeces and urine. If aromatic carboxylic acids disappear during the digestion of the mixture of faeces and urine, they are not likely to be important contributors to the odour from stored slurry.

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