Identification of Key Odour Components in Pig House Air using Hyphenated Gas Chromatography Olfactometry

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ABSTRACT

The exposure to odorants from animal production facilities creates a serious problem in the form of odour annoyance for the surrounding society and thus for the agricultural industry. The identification of the key odour components responsible for the odour annoyance is important in the search for odour reducing methods and could be useful for quantitative purposes as well.

The objective of the present study was to investigate the application of the hyphenated gas chromatography olfactometry technique (GCO) called Detection Frequency Analysis for the identification of key odour components in an odorous air sample collected in a grower/finisher pig house. Detection Frequency Analysis is based on a combination of gas chromatography and a human panel sniffing to odours eluting from the gas chromatographic column.

The human sniffing panel detected 25 odours from a grower/finisher pig house air extract. Subsequent gas chromatography mass spectrometry (GC-MS) analysis resulted in the identification of propanoic acid, 2-methylpropanoic acid (iso-butanoic acid), butanoic acid, octanoic acid, 4-methylphenol (p-cresol), indole, and possibly skatole. The odour components identified in the present study are in agreement with published data on key odour components. Many of the odours found by the odour panel could not be positively identified by GC-MS, which suggests that the olfactory sense of the human subjects may have been more sensitive than the applied GC-MS method.

The method applied in the present study does not take into account possible interactions between odour components. Validation of the identified key odour components may be performed by comparing a recombined odour based on a mixture of the identified key odour components and a real odour sample.

Further studies will reveal if additional key odour components can be identified using GC methods and if different animal categories and/or housing systems differ with regards to key odour components.

Keywords: pig house odour, key odour components, Detection Frequency Analysis, GC-Olfactometry, GC-sniffing.

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INTRODUCTION

Annoyance caused by odour from animal production facilities is a problem for the agricultural industry in relation to the surrounding society. In order to solve this problem proper methods to identify and quantify odorous components of annoyance are needed.

Identification and quantification of the volatile organic components (VOCs) in a pig house is an extremely difficult and cumbersome task. Furthermore, little information is provided about how the mixture is perceived by the human olfactory sense. Nor does it reveal the relative influence of the individual constituents of the complex mixture to the odour perception since the sensory properties between VOCs vary immensely.

Identification and ranking of odour active components in pig houses could be performed using the human olfactory sense parallel with an electronic detector. These are referred to as *gas chromatography olfactometry* (or sniffing) (GCO or GC-sniffing). Several GCO methods have been developed and applied, especially within food science. The methods can generally be categorized into three groups: *extract dilution methods*, *intensity methods, and the detection frequency method*. The dilution methods are based on sensory evaluations of stepwise dilutions (typically 1:2 to 1:3) of an aroma extract until no odours is perceived Acree, 1984; Grosch, 1993). Ranking of the components is based on the assumption, that the higher the dilution at which a compound can be detected by GCO, the more significant is the odour component. The intensity methods use human subjects to assess the intensity of eluting odour components in an aroma extract, which has been injected into the GCO (McDaniel et al., 1990; Tønder et al., 1998). The Detection Frequency Analysis (Pollien et al., 1997) is based on the assumption, that the relative number of subjects detecting an odour at any given retention time during a GCO run reflects the relative importance of the odour component. In contrast to the dilution methods, the detection frequency analysis involves only one concentration level. Instead of multiple replicates using the same few individuals, usually only two, this method prescribes the use of a panel consisting of six to ten subjects, who note down any detected odours eluting during a GCO run.

Petersen et al. (2002) found that the ranking of identified odour components was affected by the applied GCO method. The AEDA and CHARM analysis resulted in a different relative ranking of the key odour components compared to Detection Frequency Analysis and Posterior Intensity Method. The observed differences was explained by differences in the Odour Active Value[1] of the components as well as differences in the persistency[2] of the individual odour components, i.e. the rank of an odour active component may depend on the concentration level of the odour components in the sample. This finding strongly suggests that the choice of GCO method depends on the context. Thus, the identification of key odour components in odorous air from a pig house and from the highly diluted odorous air found at the borderline of an animal production facility may not result in a match.

The purpose of the present study was to demonstrate the potential for applying hyphenated gas chromatography olfactometry techniques in environmental odour research. For this purpose the *Detection Frequency Analysis* developed by Pollien et al. (2001) was employed due to its simplicity. No training of the human subjects is necessary, and it is less labour-

[1] Odour Active Value is defined as the ratio between odorant concentration and the odour detection value.
[2] Persistency is defined as the rate of change in the perceived odour intensity caused by a change in the odour concentration (or odorant concentration).

intensive, because less replicates is necessary. Furthermore the method overcomes some of the statistical shortcomings of other GCO methods. The samples were collected in a grower/finisher pig house. In doing so the analytical problems related to the dilution of the odour components in the atmosphere was abandoned.

MATERIALS AND METHODS

The air samples for odour analysis were collected in a grower/finisher pig house with the following dimensions: 12 x 18 x 2.4 m (width, length, height to ceiling). The house contained 20 pens in two rows. Each pen had a capacity of 15 pigs corresponding to a total capacity of approximately 300 pigs. On the day of sampling the house contained 204 pigs with an estimated average weight of 70 kg. The entire pen floor consisted of cast iron elements with slots (i.e. fully slatted floor) and manure pits below with a storage capacity of 3-6 weeks. The pens were equipped with troughs for simultaneous administration of liquid feed to all pigs in two pens. The house was equipped with a mechanical ventilation system with a row of air inlets in both façades and three air outlets mounted in the roof. The ventilation system operated at full capacity, corresponding to approximately 36,000 m^3/h.

Sample collection

A total of nine air samples were collected in the centre of the grower/finisher pig house at a height of 1.5 m above floor level. The samples were collected using sorbent traps made of 49.4 mm stainless steel tubes with an internal diameter of 6.4 mm. The traps contained 72 mg of Tenax-TA 60/80, 92 mg of Carbopack B 60/80 and 120 mg of Carboxen 1003 40/60. The sorbent traps were conditioned at 300°C for 30 min and concurrently flushed with ultra high purity helium carrier gas at 100 ml/min prior to sampling. Sampling was performed at a flow rate of 110 ml/min for 30 min. The nine samples were collected in succession. After the sampling, the sorbent tubes were capped and stored overnight at 5ºC.

Sample preparation

Each sorbent tube was eluted with 10 drops of diethyl ether. The eluates were pooled and concentrated to approximately 185 µl by gently blowing nitrogen gas over the surface of the sample, thereby allowing the diethyl ether to evaporate.

Detection Frequency Analysis

The detection frequency analysis on grower/finisher pig house odour was conducted using a gas chromatograph equipped with a sniffing port (GCO) and a human sniffing panel. The GC was an HP 5890 series II Plus gas chromatograph (Agilent Technologies, CA, USA) equipped with a DBWAX GC column (30 m, 0.25 mm internal diameter, 0.25 µm stationary phase) (J&W Scientific, USA). Ultra high purity helium gas was used as carrier gas at a flow rate of 1.0 ml/min. One µl of the sample was injected using splitless injection at 250˚C. The initial oven temperature was 45˚C increasing to 240˚C at 10˚C/min. The GC was equipped with an ODO-1 glass sniffing mask (SGE, Victoria, Australia). The effluent from the GC column was mixed with 150 ml/min of carrier gas, which was humidified with demineralised water prior to exposure to the human subjects.

The sniffing panel consisted of seven human subjects, four of whom were trained GCO judges, while the others were untrained. None of the subjects were screened on odour components associated with the present topic prior to the analysis. During analysis, the subjects recorded the time for onset and end of a perceived odour while sniffing the effluent

from the sniffing mask. When possible the subjects also made notes about the character of the eluting odours. The odour sample was evaluated once by each of the seven human subjects. On the basis of the GCO evaluations, an aromagram was constructed having the Nasal Impact Frequency as a function of time (NIF_t):

$$NIF_t = N_t/n \cdot 100 \quad [1]$$

where N_t is the number of subjects recognizing an odour at time t, and n is the total number of subjects exposed to the GCO effluent at time t.

A NIF_t score of 100% means that an odour was detected by all n subjects at a certain retention time, i.e. the concentration of the odour component exceeded the odour detection threshold for all subjects. The lowest possible NIF_t ($NIF_t = 100/n$) corresponds to an odour component that was detected by only one of the subjects (Pollien et al., 1997).

Identification of air sample constituents

Identification of the analytes was performed using a HP GC-MS model 6890/5973 (Agilent Technologies, CA, USA) equipped with a GC column identical with the GCO and applying the same analytical procedure as the GCO. The mass spectrometer detected components between 35 and 300 m/z (ion mass/charge ratio). Identification was based on the National Institute of Standards and Technology (NIST) MS spectral library.

GCO-GC synchronization

Since the sensory evaluation and the identification of the odorants in the odour sample was performed on different analytical equipment, the identification of the odorants resulting in the perception of odours by the subjects required synchronization. To accomplish that the retention times of the aromagram and chromatogram produced on the GCO and GC-MS, respectively, were synchronized using a standard mixture of odour components, which was analyzed using both GC systems. One µl of the standard odorant mixture was analyzed on the GC-MS system and subsequently on the GCO. The retention time obtained for each odorant was plotted against each other and a linear equation was derived using the Microsoft Office Excel 2003 software package (Microsoft Inc., USA). As a result, the retention time of an odour obtained on the GCO system could be correlated with the retention time of the same odour on the GC-MS.

RESULTS AND DISCUSSION

Four odours were detected by all seven subjects, three odours were detected by six subjects, nine odours were detected by five subjects, and three odours were detected by four subjects (Figure 1). The lowest NIF_t score (100/n; i.e. 0.14) was an odour detected by a single subject indicating either “odour noise” due to external odorants or components below the detection threshold for all of the other subjects (Pollien et al., 1997).

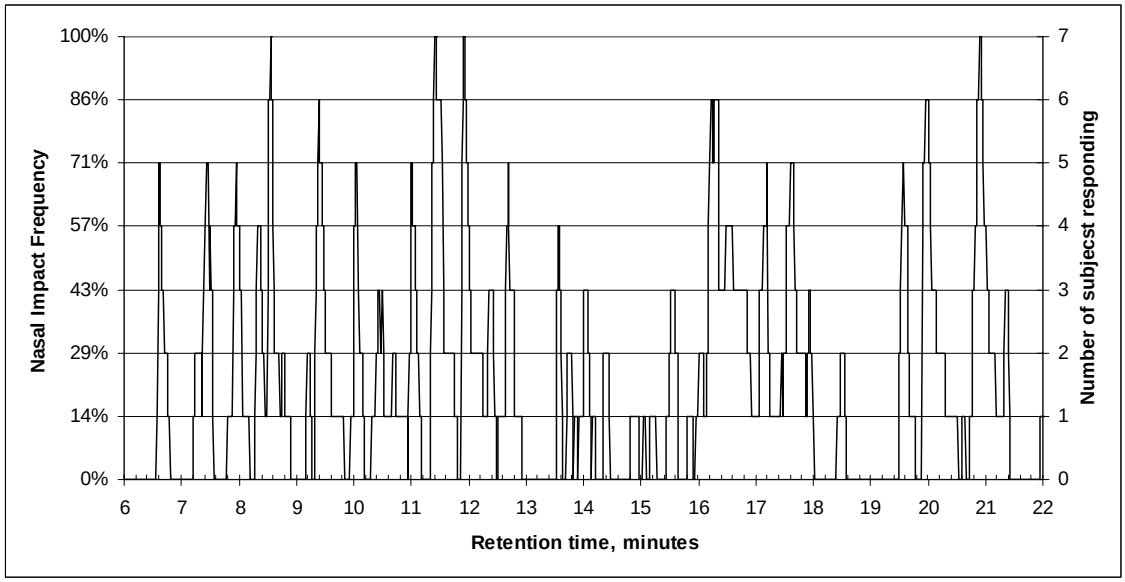

Figure 1. Nasal impact frequency / number of subject responses as a function of time after injection of the pig house odour extract. The odour panel consisted of seven subjects.

Qualitative analysis employing GC-MS resulted in the positive identification of 17 components. Since the air inside a pig house contains several hundred VOCs (Schiffman et al., 2001), this finding was surprisingly low. One explanation may be that the solvent extraction method using diethyl-ether was inadequate in desorbing the components retained in the sorbent matrix. The concentration step involving purging of the surface of the sample with nitrogen carrier gas may also have caused the more volatile species to vaporize. Kim-Yang et al. (2001) described a similar lack of sensitivity when performing an even more extensive solvent extraction (also using diethyl-ether) of sorbent trap samples collected in a nursery pig facility.

Synchronization of retention times

A high linear correlation between retention times on the GC-MS and the GCO was found (Figure 2). The observed consistent retention time delay of 39 s in average observed for the GCO is caused by differences in the analytical equipment. Furthermore, the detection of odours by the olfactory epithelium in the nasal cavity requires time to be processed in the limbic system in the brain. This processing time appears as a response time when comparing the retention times of a GCO and a GC-MS.

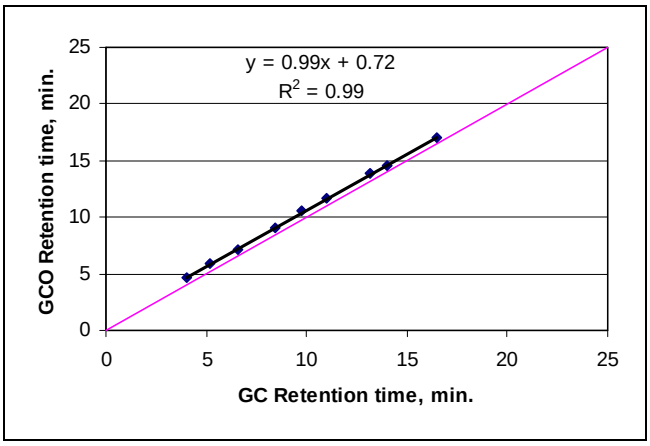

Figure 2. Synchronization of GC-MS GCO retention times. Each dot represents an analyte.

The detection of odours during a GCO run depends on the breathing cycle of the human subjects so is not a continuous process. Some analytes may elute during human exhalation resulting in either lack of detection of the odour or in an additional GCO retention time delay compared with the GC retention time. Since the GCO retention time is not a true point in time, but a time period during the elution of an odorant, where the odour detection threshold concentration of the analyte is exceeded, the GCO retention time may precede the GC retention time. This phenomenon is depicted in Figure 3.

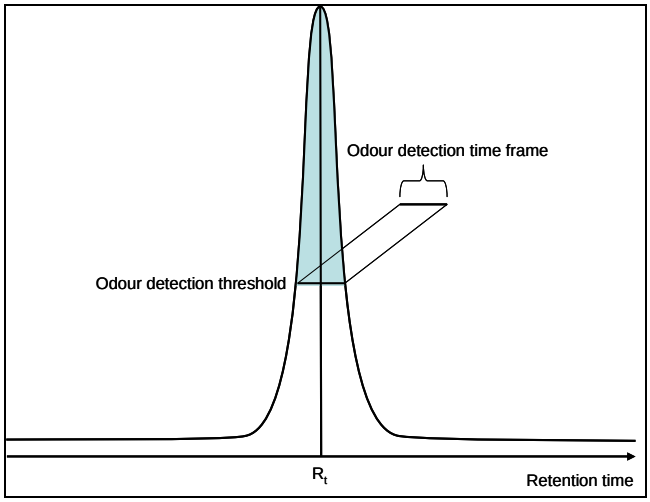

Figure 3. Variation in GCO retention time in relation to the GC retention time. Rt = GC retention time.

Note that in many GC systems it is possible to install a sniffing mask parallel with the electronic detector using a column splitter. In such cases the synchronization step could be optimized.

Identification of high NIF_t odours

The NIF_t scores and the GC-MS results have been tabulated for comparison (Table 1). 25 odours were detected by at least three of the seven subjects and are thus associated with NIF_t

scores of 43% or higher. The GC-MS analysis resulted in the identification of 17 components. Six of these components could be directly associated with odours detected by the subjects. The components were *propanoic acid, 2-methyl propanoic acid (iso-butanoic acid), butanoic acid, 4-methyl phenol (p-cresol), octanoic acid, and indole*. Other odours detected by the subjects, but not identified by GC-MS were probably present in concentrations below the method detection limit of the GC-MS. Analytes identified by GC-MS, but not by the human subjects were present in concentrations below the human olfactory detection threshold, or they were not odour active components.

Table 1. Comparison of GCO observations with components identified by GC-MS. Odours and odorants appear in order of retention time. Only NIF_t (Nasal Impact Frequency) scores of 43 % or higher are included. Shaded areas indicate a GCO – GC-MS match.

NIF_t pct.	GCO, retention time, min.	Panel descriptions	GC-MS retention time, min. [1)]	Identification
71	6.61			No ID
71	7.45	Mushroom		No ID
71	7.93	Boiled rice		No ID
57	8.35			No ID
100	8.56	Pig odour, forest floor		No ID
86	9.40	Forest floor		No ID
71	10.05			No ID
43	10.46		10.44	Propanoic acid
71	11.01	Sour	10.93	2-methyl-propanoic acid
100	11.42	Sweat, sour, pig house, cheese	11.49	Butanoic acid
100	11.92	Sour, pig house, feet		No ID
			12.11	3-methyl-butanoic acid
43	12.39	Feet		No ID
71	12.68	Sour, unpleasant, cheese		No ID
			12.74	Methoxy-phenyl-oxime
			12.88	Pentanoic acid
57	13.57			No ID
43	14.05	Burnt, tar		No ID
			14.34	Butylated Hydroxytoluene
43	15.56		15.47	Phenol
86	16.28	Pig house, faecal	16.21	4-methyl-phenol
57	16.54	Pig house	16.39	Octanoic acid [2)]
			17.09	4-ethyl-phenol
71	17.20	Tar		No ID
71	17.63	Faecal		No ID
43	17.94	Tar		No ID
			18.94	Hexadecanenitrile
71	19.59	Pig house	19.55	Indole
86	19.98	Faecal, pig house, unpleasant		No ID
100	20.91	Flower, fresh, fermented, sour		No ID
43	21.37			No ID
			21.74	Dibutyl phthalate
			22.27	Tetradecanoic acid
			26.13	Hexadecanoic acid
			29.67	Bis(2-ethylhexyl) phthalate

1) Retention time normalised according to Figure 2.
2) Dubious identification, due to possible co-elution of two or more components.

Note that of the four odours with a NIF_t score of 100%, only butanoic acid was positively identified, and of the three odours with a NIF_t score of 87%, only 4-methyl-phenol was identified. Air samples analysed by GC-MS from the grower/finisher pig house collected using solid phase microextraction (SPME) and sorbent trapping (Kai, preliminary data, 2004) suggest that the unidentified odorant eluting at retention time 19.98 min, described as "faecal", "pig house" and "unpleasant" was most likely skatole (3-methyl indole).

The odour descriptions noted by the odour panel agree with the assumption that the odour components associated with these retention times may be important key odour components associated with pig house odour (Table 2). Some variation exists in the content between the published odour compositions, possibly due to differences in animal housing and diet composition, and because odours from pig houses and pig manure differ. The sampling method is determinative for the components retained from the air, the desorption efficiency, and the GC/MS parameters are determinative for the sensitivity of the analysis.

Table 2. Key odour components associated with pigs and pig manure.

Odour component	Pig house odour				Pig manure odour			
	Present study	Previous studies: Reference No.						
		1	2	3	4	5	6	7
Acetic acid		X	X	X	X			
Propanoic acid	X	X	X	X	X			
i-butanoic acid	X		X	X		X		X
Butanoic acid	X	X	X	X	X	X	X	X
iso-pentanoic acid			X	X	X	X	X	
Pentanoic acid			X	X	X		X	
i-hexanoic acid			X	X				
Hexanoic acid			X	X				
Heptanoic acid			X	X				
Octanoic acid	X			X				
Nonanoic acid				X				
Phenyl acetic acid		X						
3-phenyl propanoic acid		X						
Indole	X		X	X	X	X	X	X
Skatole	(X)		X	X	X	X	X	X
Phenol		X	X	X	X		X	X
3-methyl phenol							X	
4-methyl phenol	X		X	X		X	X	X
4-ethyl phenol		X	X	X			X	
2-butanol			X			X		
3-methyl butanol					X	X		
Phenyl methanol			X					
Phenyl ethanol							X	
Dimethyl disulfide			X		X	X	X	
2-amino acetophenone			X					
3-hydroxy-2-butanone					X			
2,3 butanedione					X			X
Pyrazine				X				
2-methyl pyrazine				X				
2,3,4,5 tetramethyl pyrazine				X				

Sum key odour components	7	18	19	12	9	11	7

References: [1]Hammond et al. (1979), [2]Zahn et al. (2001[a]), [3]Oehrl et al. (2000), [4]Burnett (1969), [5]Yasuhara et al. (1980), [6]Cassé & Baker (1996), [7]Schäfer et al. (1974) cited from O'Neill & Phillips (1992).

It is important to realize that the results obtained using a single GC method, e.g. GC column, may not result in the detection of all VOCs in the sample, and thus may not reveal all key odour components. In the present study, no sulphur components were identified, which may have been due to the analytical equipment or the sampling proceedure. Thus, it is recommended to employ more than one analytical method, e.g. two GC columns with different polarity, in the attempt to cover the full range of odour active components in the material of interest. Also, much attention should be paid to the sample collection, since this step is crucial for later recovery and identification of the analytes and thus key odour components.

Effects of interaction between odorants on the human olfactory perception have been reported (Zahn et al., 2001[b]). Since the identification of key odour components using GCO methods is based on the concept of chromatographic separation of the individual odorants in a time continuum, GCO methods cannot account for possible interactions between odour components. Thus, GCO methods may not result in the detection of all of the key odour components. Validation of the identified key odour components may be performed by recombining the odour based on a mixture of the key odour components and compare it with the true odour mixture.

Further studies will reveal if other key odour components can be identified using GCO methods and if the analysis between different animal categories and housing systems differ.

CONCLUSION

The Detection Frequency Analysis method resulted in the detection of 25 odours with a NIF_t score at or above 43%. Of these, *propanoic acid, 2-methyl-propanoic acid, butanoic acid, 4-methyl-phenol, octanoic acid, indole,* and possibly *skatole* were identified. The GC-MS analysis did not reveal the identity of the remaining 18 odours detected by the human panel. The odour descriptions noted by the odour panel agree with the assumption that the found odour components may be important key odour components associated with pig house odour.

Since the identification of key odour components using GCO methods is based on the concept of chromatographic separation of the individual odorants in a time continuum, GCO methods cannot account for possible interactions between odour components. Thus, GCO methods may not result in the detection of all key odour components. Validation of the identified key odour components may be performed by recombining the odour based on a mixture of the key odour components and compare it with the true odour mixture.

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