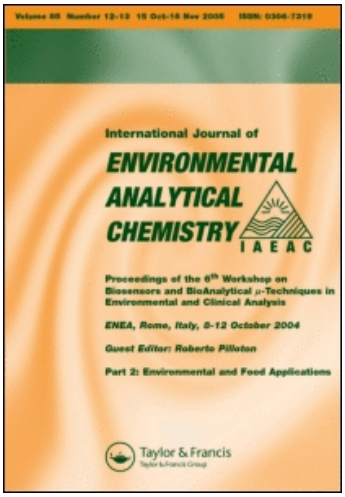

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IMPLEMENTATION OF NEW METHODS TO CHARACTERISE ATMOSPHERES IN PIGGERIES

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Two procedures to characterise atmospheres in piggeries are presented. The first allows delocalised sensorial analysis by trapping volatiles from pig shed emissions in a lipid phase, followed by laboratory analysis of their odour characteristics. The second procedure provides instrumental odour signatures of atmospheres. The volatile compounds are concentrated by solid-phase microextraction (SPME), and analysed directly by mass spectrometry without a chromatographic step (SPME-MS). The information supplied by the two analysis methods proved rich and consistent for the 42 piggeries analysed. In addition, the odour signatures allowed a good estimation of the key dimensions of the odour of the lipid phases. This result indicates that SPME-MS is a promising instrumental method to estimate the degree of odour nuisance in livestock buildings.

Keywords: SPME-MS; Spectral signature; Sensorial analysis; Odour nuisance

INTRODUCTION

The adverse environmental impact on soil, water and air of intensive pig farming is well recognised [1]. Unpleasant odour emissions, which can be a particular nuisance at up to several kilometres from the point of emission [2], are a major criterion governing the installation of new piggeries. Although there are technical solutions for constructing livestock buildings that limit and recycle their liquid and gaseous effluents with practically no environmental impact, political and economic conditions favouring their implementation are currently lacking. Consequently, a better characterisation of odour emissions is currently necessary to identify and describe those sites that present the highest nuisance risks. For this purpose sensory and instrumental approaches are parallel and complementary. The direct use of a sensorial approach comes up against numerous technical and financial limitations, e.g., on-site provision of a sensory analysis jury, dependence on weather conditions, and rapid saturation of judges in

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an excessively odorous atmosphere [3]. Similarly, the standardised measurement of an olfactive dilution threshold from a sample of a fraction of the atmosphere studied collected in a balloon (AFNOR Standards NF X 43-10, NF X 43-103 and NF X 43-104) presents many limitations. Besides the fact that it neglects the qualitative dimension of the atmosphere studied, its use of bagged samples brings problems of adsorption of volatiles and condensation of water, both sources of marked analytical artefacts [4]. In addition, the results of the instrumental approach have proved disappointing, and the numerous studies conducted on the subject have served only to emphasise the limits of the classical analytical approaches [3,5–10]. However, new procedures can be considered, using relatively simple and rapid methods. Solid-phase microextraction (SPME) is a recent method for the trapping of volatile organic compounds (VOCs) that has already been applied to environmental monitoring [11]. Coupled to gas-phase chromatography and mass spectrometry (SPME-CPG-SM), it provides detailed information on the volatile fractions of matrixes or atmospheres [12–14]. Coupled directly to mass spectrometry, i.e., with no prior chromatographic separation, SPME-MS rapidly yields mass spectra characteristic of the overall composition of the VOCs [15–18].

The aim of this work was to implement two simple and rapid procedures for the characterisation of piggery atmospheres. The first consisted of trapping the volatile compounds from pig sheds in a lipid phase, followed by laboratory analysis of the odour characteristics. The second method involved concentrating the volatile compounds by SPME, and then analysing them directly by mass spectrometry with no prior chromatographic step (SPME-MS). It rapidly yielded odour signatures of the atmospheres. Lastly we sought to correlate the analytical results obtained by the two atmosphere characterisation procedures.

EXPERIMENTAL

Selection of Piggeries and Sampling Conditions

Forty-two piggery sites distributed over north-western France were selected so as to be representative of the broad diversity of existing pig farming conditions. Two criteria of variation were studied: ''animals' physiological state'' (suckling sows, weaned piglets or fattening pigs), and ''type of floor used'' (slatted flooring or litter). The distribution of the sites according to these criteria is presented in Table I. The sampling campaign lasted eight weeks during March and April 2002. All the samples were taken by the

TABLE I Distribution of samples according to the ''animals' physiological state'' (E: fattening pigs, PS: weaned piglets, M: suckling sows) and ''type of floor'' (C: full concrete slatted flooring or partial slatted floor, L: straw or sawdust)

	PS-C ■	*E-C* ●	*M-C* ▲	*PS-L* □	*E-L* ○
Total strength	8	13	7	6	8
Mean odour intensity scores assigned by the experimenter during on-site sampling	6.8 ± 0.8	7.2 ± 1.2	5.4 ± 0.8	3.2 ± 0.8	4.6 ± 1.6

same experimenter, who also undertook a field survey on the characteristics of each installation: cleanliness of buildings, manure management, mode of ventilation and odour intensity score, from 1 (very weak) to 10 (very strong). For each piggery site the samples for sensory analysis and instrumental analysis were taken simultaneously inside buildings at 1 m above floor level and away from areas of air turbulence (doors, fans, extractors, etc.).

Sensorial Analysis

Preparation of Absorbents – Absorption of Volatile Compounds

The absorbent was made of 25 g of lipid phase (deodorised lard, SARL R. Bahier, Sceaux-sur-Huisne, France) cast in a glass Petri dish (diameter 8 cm). This deodorised fat offers the advantage of having a very weak odour and being sufficiently viscous at ambient temperature not to flow. It is also a powerful absorbent of piggery odours and does not become perceptibly rancid during analysis. For each piggery a sufficient number of Petri dishes for subsequent sensory analysis requirements were exposed for 120 min. The dishes were then sealed airtight, frozen at $-20^{\circ}C$ and transferred for laboratory analysis within three days.

Sensory Testing

A panel of ten assessors was formed (seven men and three women, aged 25 to 55 years). Beforehand, the assessors tested lipid phases that had been exposed in seven sites representative of the olfactive diversity of piggery atmospheres. Based on these tests an open discussion was held to eliminate non-consensual or redundant descriptors. Nine sensory descriptors were left: "Piggery", "Faecal", "Urine", "Cabbage-Gas", "Rind", "Rancid-Fatty", "Manure-Stable", "Straw" and "Mushroom". The descriptor "Global Intensity" was added to this list. The olfactive analysis of the lipid phases from the 42 piggery sites was of the profile type. Eight test sessions were organised. At each session, five or six closed Petri dishes (warmed to ambient temperature) corresponding to olfactively distinct pig farming sites were presented simultaneously and blindly to the assessors. The assessors could compare the odours of the lipid phases before scoring each descriptor on a graded scale from 1 (very weak) to 10 (very strong). The sessions lasted 10 to 15 min according to the assessors.

Instrumental Analysis

Solid-phase Microextraction of Volatiles

During each sampling procedure, three carboxen-polydimethylsiloxane (C-PDMS) type SPME fibres 75 μm thick (ref. 57344-U, Supelco, Saint-Quentin-Fallavier, France) were simultaneously exposed for 120 min inside the building. After exposure, the fibres were isolated from the atmosphere by inserting the metal ends of their syringes in septum caps (ref. 5181-3383, Agilent Technologies). The fibres were then taken to the laboratory at ambient temperature and analysed within three days. In all, 126 samples were taken (3 fibres per site $\times$ 42 piggeries).

SPME-MS Measurements

The analytical set-up consisted of a gas-phase chromatograph (Model 6890 A, Agilent Technologies, Palo Alto, CA) coupled to a quadripolar mass spectrometer (Model 5973, Agilent Technologies, electron impact ionisation at 70 eV). The volatile compounds adsorbed on the C-PDMS fibres were desorbed in the splitless injector of the chromatograph for 2 min at 280°C and then transferred directly into the mass spectrometer ionisation chamber through a short transfer line heated to 220°C (HP-retention gap ref. 19091-60620, Agilent. Length 3 m, diameter 0.10 mm). In these conditions, the analysis time was 5 min. The range of masses acquired was $33 < m/z < 250$ amu. The total ion current thus obtained had the form of an asymmetrical peak of duration 5 min and mid-height width between 0.25 and 0.50 min. The average mass spectrum, derived from simultaneous ionisation and fragmentation of all the molecules injected, was calculated. This average spectrum was taken as the crude odour signature of the atmospheres analysed.

Pre-treatments and Statistical Analysis

All the statistical calculations were performed with Statistica software [19].

Sensory Data

To describe the variability of the nine olfactive descriptors, the mean, the median and the interquartiles of the scores of the ten assessors were calculated for the 42 pig farms considered. The pig farms were classified by ascending hierarchical clustering (AHC on Euclidian distance with aggregation of individuals by Ward's method [20]). The aggregation distance was expressed as a percentage of the greatest inter-cluster distance observed. A detailed study of the olfactive characteristics of the different piggery sites was conducted by principal components analysis (PCA). To conserve the information linked to the descriptor scores the PCA was performed from the variance–covariance matrix.

Instrument Data

Out of the 218 mass fragments that made up the spectra, those with m/z values less than 45 amu (multiple or poorly characteristic origins: ambient air, carrier gas) together with those covered by instrument noise (estimated at 10^5 arbitrary abundance units according to the method of Begnaud and Berdagué [21]) were discarded, leaving spectra containing only 104 mass fragments. These spectra were normalised before statistical analysis (every fragment was expressed as a percentage of the total abundance of all the mass fragments). This mode of expression eliminates variations in response level linked to fluctuations in the mass detector sensitivity or variable adsorption levels of the volatile compounds on the SPME fibre [15]. Median filtering was then applied to the three repeats of each site to stabilise the variance of the measures [22]. A synthetic representation of the data was obtained by PCA of the median spectral data (correlation matrix).

Estimation of Sensory Characteristics from Instrument Data

The estimation of sensory data was made by multivariate linear regression with prior selection of a relevant subset of mass fragments per stepwise ascending procedure ($p < 0.05$).

RESULTS AND DISCUSSION

Olfactive Analysis of Lipid Phases

The method of trapping of volatile emissions on a lipid phase proved very easy to implement; all that had to be done was to open the Petri dish and place it at a set location to trap the odours. After trapping, the odour of the phase was qualitatively very close to that of the piggeries but always less intense. Pre-tests showed that by smelling the lipid phases exposed in ''strongly odorous'', ''moderately odorous'' and ''mildly odorous'' atmospheres the different piggery sites could be easily distinguished and classified. All these observations supported the choice of the lipid absorbent. One of the advantages of odour assessment on a lipid phase, besides allowing the assessment to be delocalised, is that the sampled odours are less intense than those perceived directly in the piggery atmospheres, which often saturate the assessors' sense of smell.

The results of lipid phase odour assessment exposed in the 42 piggeries confirmed that the method is sensitive. The odour of the lipid phases differed widely according to the atmosphere. The most widely ranging descriptors (Fig. 1) were: ''Global intensity'' (max. 6.9, min. 1.2), ''Piggery'' (max. 5.2, min. 0.1) and ''Faecal'' (max. 5.2, min. 0). The other descriptors varied much more narrowly, in particular the

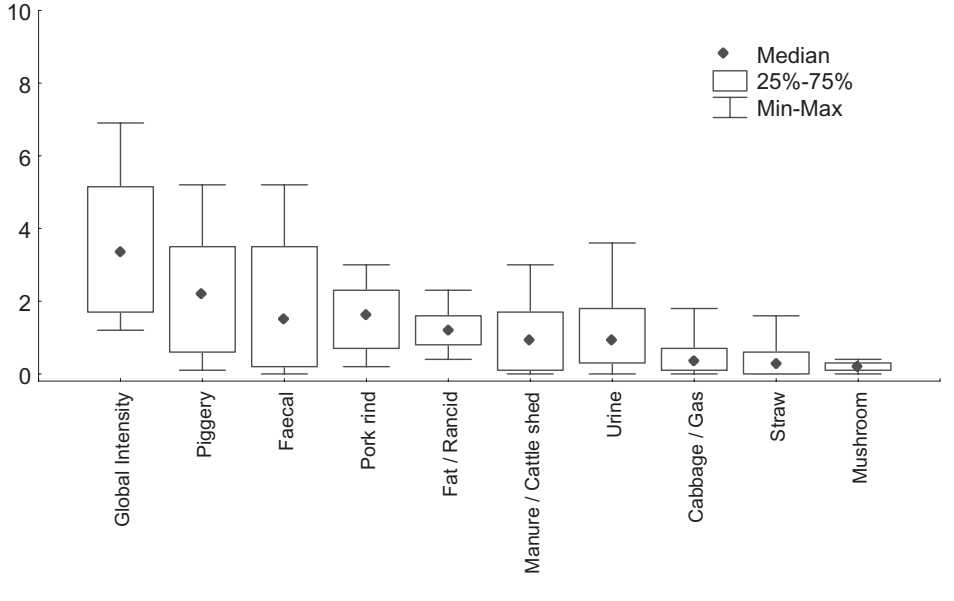

FIGURE 1 Mean, median and interquartile of sensory descriptors.

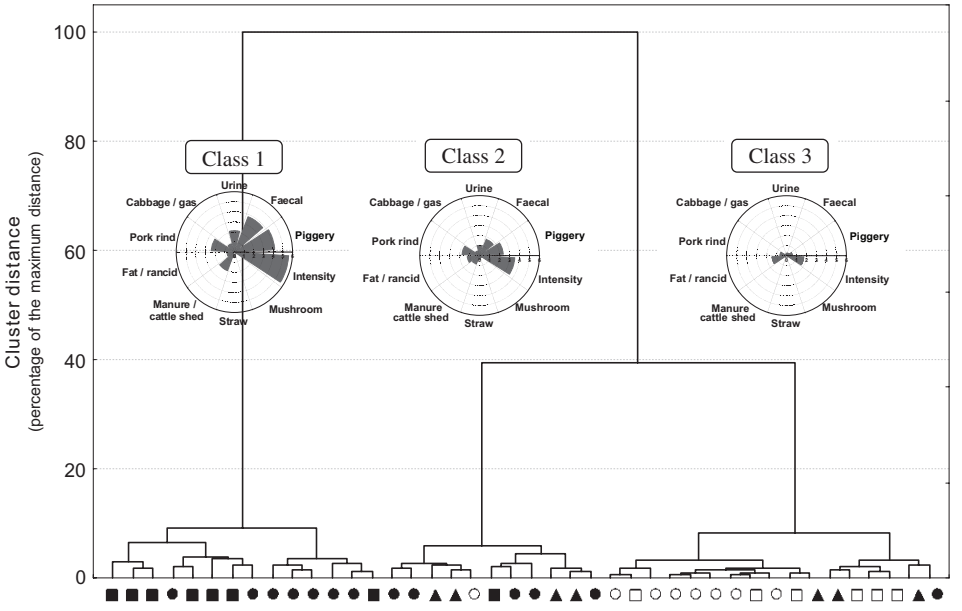

FIGURE 2 Classification tree for piggeries according to olfactory data (ascending hierarchical clustering) and sensory profiles associated with the three clusters found. The aggregation distance is expressed in percentage of the greatest inter-cluster distance measured. Rearing conditions: ■ = weaned piglets on slatted flooring, □ = weaned piglets on litter, ● = fattening pigs on slatted flooring, ○ = fattening pigs on litter, ▲ = suckling sows on slatted flooring.

"Mushroom" descriptor, the intensity and variability of which were very low. In practice this descriptor thus proved unrepresentative of the odour of fatty phases.

The ascending hierarchical clustering of the 42 odour assessments revealed three classes of olfactive perception (Fig. 2). The sensory characteristics of each class were presented in the form of polar diagrams termed "sensory profiles" (Fig. 2). These profiles corresponded to average profiles calculated from the *n* piggeries in each group. The classification shows that Class 1 stands out sharply from Classes 2 and 3. Class 1 groups the most strongly odorous lipid phases, and according to the observations made in the piggeries by the technician responsible for the sampling, it also corresponded to the 14 most strongly odorous piggeries (pigs on slatted floors, Table I). In contrast, the 17 piggeries grouped in Class 3 (pigs on straw litter or sawdust) corresponded to the least intense olfactive perceptions during odour assessment of lipid phases. The atmospheres in the piggeries in Class 3 were mildly odorous. Class 2 grouped the moderately odorous lipid phases. This last class corresponded to the 11 piggeries, mostly with slatted floors, that presented appreciably less intense odours than Class 1 piggeries.

The survey of the piggeries' distribution (main plane of the principal components analysis, Fig. 3a) shows that the odour assessment on the lipid phase was able to separate the piggeries according to the nature of the flooring used for the pigs. From left to right, Axis 1 clearly differentiates most of the piggeries using slatted flooring (intense odours on site) from those using litter (mild odours on site). However, no distinction could be made between full slatted concrete flooring and part slatted flooring, or between straw litter and sawdust. It is interesting to note

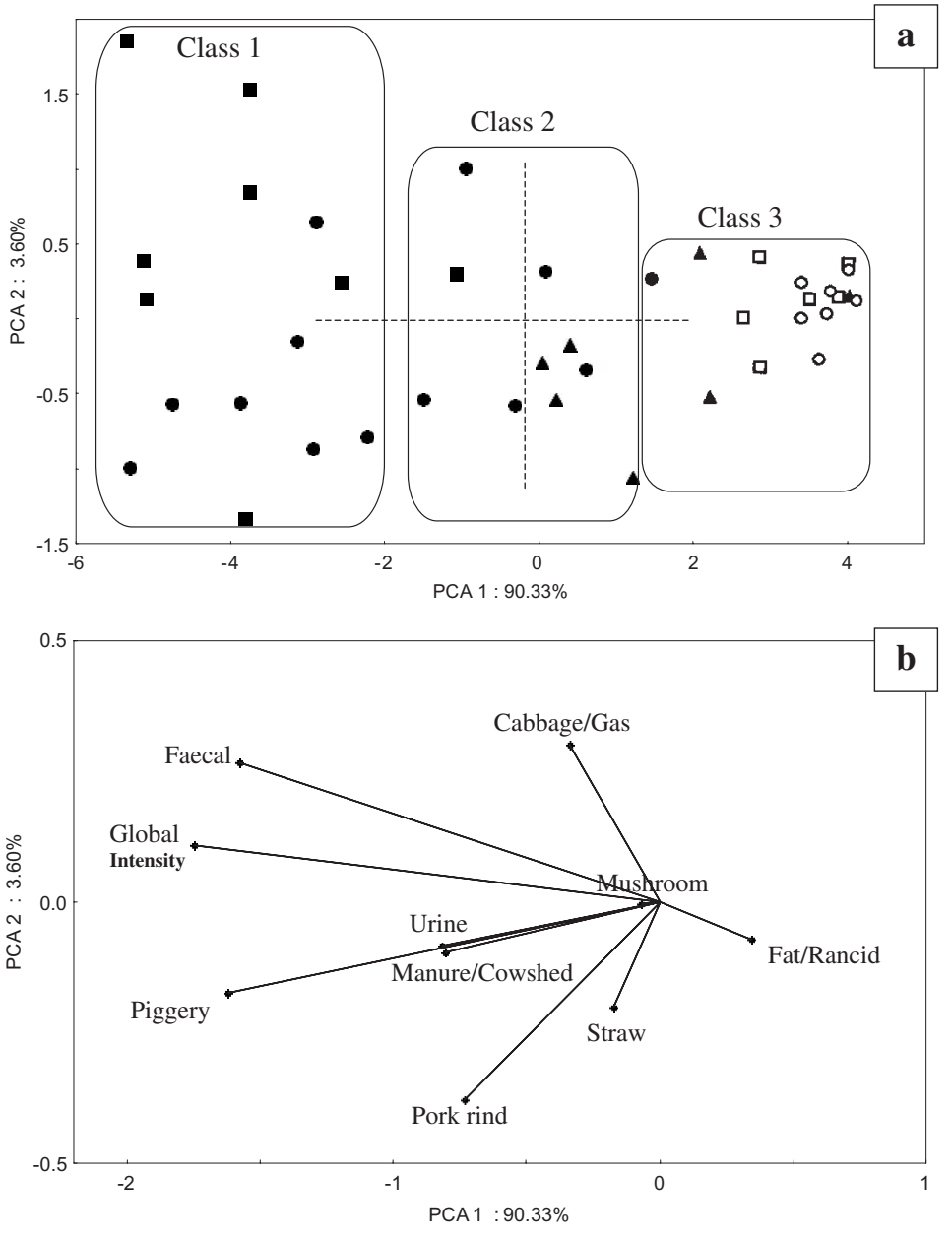

FIGURE 3 Principal components analysis (PCA) of the olfactory data: scores (a) and loadings (b). The three clusters revealed by the AHC are presented. Rearing conditions: ■ = weaned piglets on slatted flooring, □ = weaned piglets on litter, ● = fattening pigs on slatted flooring, ○ = fattening pigs on litter, ▲ = suckling suckling sows on slatted flooring.

that suckling sheds, recognised as moderately odorous, are scored close to straw litter in spite of the use of slatted floors. The field survey conducted provided an explanation for the position of several piggery buildings that were badly classified in the schematic description made above. Four piggeries on slatted floors were

assigned to Class 3, among piggeries using litter (Fig. 3a). These were three particularly clean suckling sheds and a fattening shed with a very small amount of manure in the pits when the sampling was being done. These sheds exhibited a very mild overall odour. Likewise, the piggery using litter classified among those on slatted floors (Class 2, Fig. 3a) was using a poor-quality odorous litter. Figure 3(b) shows the aromatic dimensions perceived during odour assessment of the lipid phases. As the PCA loadings were calculated from the variance–covariance matrix, the standard of the vectors associated with the descriptors is a direct measure of their intensity. Three important descriptors emerge: ''Global Intensity'', ''Faecal'' and ''Piggery''. The other descriptors had a smaller vectorial standard, attributable to lower scoring. The ''Manure-Stable'' and ''Urine'' descriptors were co-linear with the ''Piggery'' descriptor and give no real additional information. The poor representation of the descriptors ''Mushroom'', ''Straw'', ''Cabbage-Gas'' and ''Rancid-Fatty'' is linked to their low scores and narrow variances (Fig. 1). Part of the ''Rancid-Fatty'' descriptor corresponds in fact to the baseline odour of the lipid phase (perceptible at an intensity score of about 1), which, after exposure in an odorous site is masked by the volatile compounds that impregnate the support. The high variance of the main plane of the PCA (93.9%; more than 90% for Axis 1) reflects the one-dimensional aspect of the sensory perceptions, which are summarised by ''Global Intensity'' described by the terms ''Piggery'' and ''Faecal''.

SPME-MS Measurements

The direct coupling of SPME to the mass spectrometer yielded, in under 5 min, mass spectra, or spectral signatures, characteristic of the overall composition of the VOCs. The information contained in these spectral signatures results from the simultaneous fragmentation of the VOC mixture in the mass spectrometer source. An example of an SPME-MS desorption profile and its corresponding spectral signature are presented in Fig. 4. The choice of an SPME carboxen-polydimethylsiloxane (C-PDMS) SPME fibre was made from the results of the study by Begnaud *et al.* [16], who showed its potential for characterising the atmospheres of livestock farming sites. This type of fibre extracted more than 100 compounds from pig manure emissions, some of which are directly implicated in olfactive nuisance: straight- and branched-chain volatile fatty acids, nitrogen heterocycles (derivatives of pyrrole, indole and scatole), thiols and mercaptans.

Principal components analysis of SPME-MS data (Fig. 5) shows that the main plane condenses only 55% of the variance of the data. Nine canonical axes have to be considered to condense 90%. This means that the information available in the signatures displays a relatively low redundancy, which is a valuable feature of SPME-MS spectra. Inspection of the main plane (scores) clearly distinguishes the sites of fattening and post-weaning on litter from those of post-weaning on slatted floors. The mass fragments (loadings) specific to compounds implicated in odour nuisance indicate that the sites of post-weaning on slatted floors were associated with a high proportion of ions specific to carboxylic acids (m/z 60, 73 and 87: acetic, butanoic and valeric/isovaleric acid) and their corresponding ethyl esters (m/z 87 and 88). Sites of fattening on litter were characterised by the spectra with the smallest proportions of ions specific to *p*-cresol and ethylphenol (m/z 107

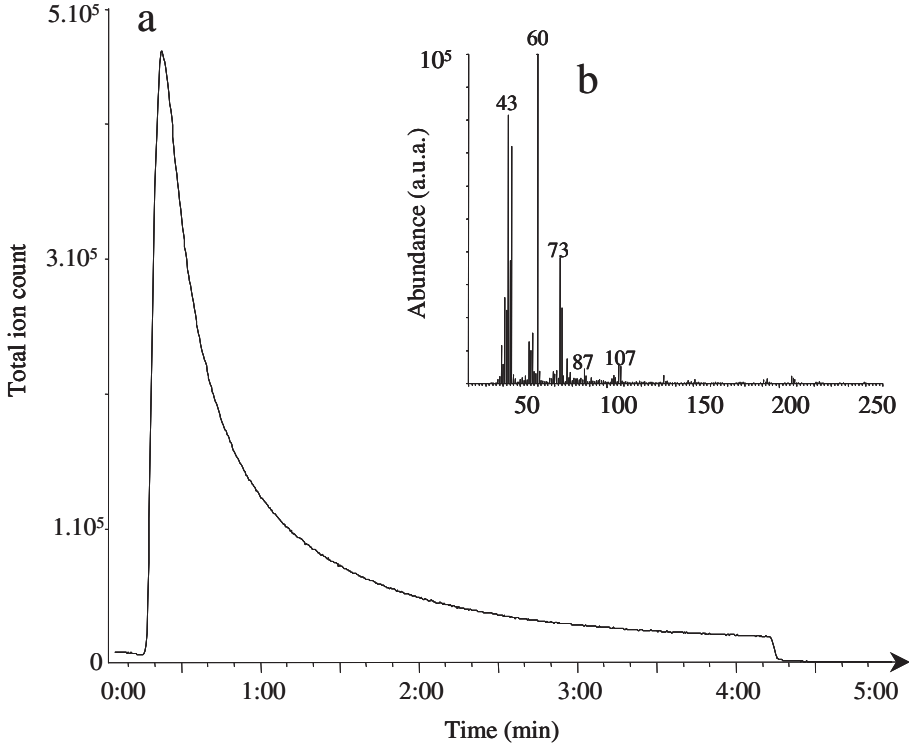

FIGURE 4 SPME-MS desorption profile of a C-PDMS fibre exposed for 120 min in a fattening shed with full concrete slatted flooring (a) and average spectrum 0–5 min (b).

and 108) and carboxylic acids, and the highest proportions of ions characteristic of terpenes (*m*/*z* 93, 121 and 136).

Estimation of Sensory Characteristics from Instrument Data

Only the sensory descriptors best perceived by the assessors were regressed. These were the descriptors "Global Intensity", "Piggery" and "Faecal", which were both scored high and displayed high variance. For these three descriptors, the model coefficients of determination (r^2) lay between 0.88 and 0.92 ($n = 42$, $p < 0.0001$) for a number of independent variables (or mass fragments) between 4 and 6 (Fig. 6). Based on separative analysis of atmosphere compositions previously carried out by Begnaud *et al.* [16], it was possible to identify the main components responsible for the occurrence of the selected fragments. They were compounds known to contribute to piggery odour [10]: sulphur compounds (dimethylsulphide, dimethyldisulphide, dimethyltrisulphide and dimethyltetrasulphide, ions 45, 79, 80, 81 and 128), volatile fatty acids (acetic, butanoic and valeric/isovaleric acids, ions 45, 60 and 87), indoles (indole and scatole, ions 87, 90 and 128), aromatic compounds (*p*-cresol and 4-ethylphenol, ions 60, 79, 80, 81, 87 and 90) and furan compounds (in particular 2-methylfuran, ion 81). The results of estimation of sensory characteristics from

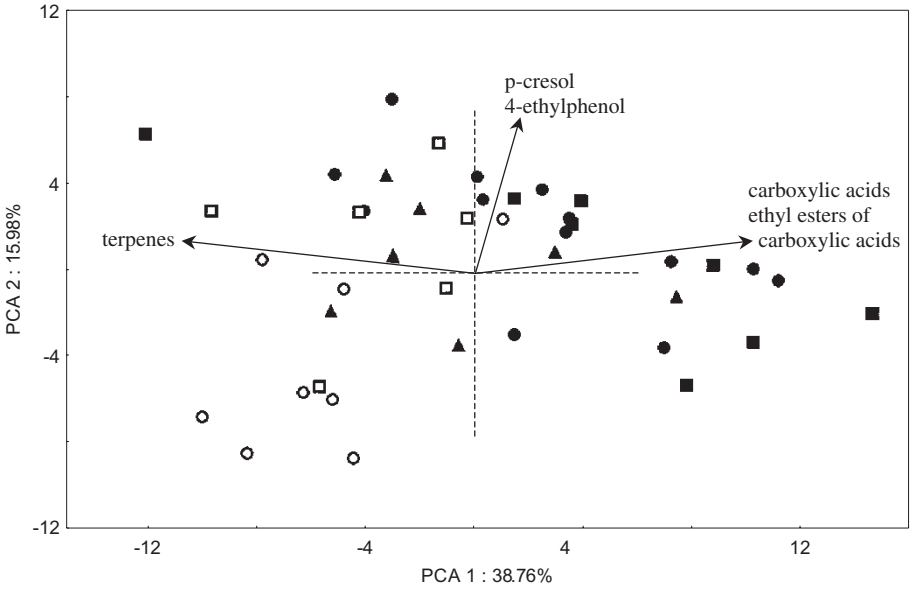

FIGURE 5 Principal component analysis (PCA) of the SPME-MS data. Biplot of scores and loadings. For simplicity, only vectors corresponding to odour-active compounds are presented. Rearing conditions: ■ = weaned piglets on slatted flooring, □ = weaned piglets on litter, ● = fattening pigs on slatted flooring, ○ = fattening pigs on litter, ▲ = suckling sows on slatted flooring.

instrument data are both highly significant and consistent with current knowledge of the molecular origins of odour nuisance. Thus odour intensity estimation by SPME-MS seems to deserve further research. Given the close links observed between subjective odour assessment data and the olfactive impressions noted on site by the experimenter (Fig. 7), it might also be interesting to attempt a direct correlation between on-site olfactive perception (using a sensory analysis assessment panel) with data acquired by SPME-MS. The ultimate aim would be to devise models for the instrumental estimation of odour nuisance in any livestock farming or industrial site.

CONCLUSION

Two methods to characterise piggery atmospheres were implemented.

The first involved collecting emissions on piggery sites by trapping volatiles in a lipid phase. This allows the olfactive characteristics of pig farm buildings to be evaluated remotely in a sensory analysis laboratory and so obviates the presence of sensory panellists on site. This method is simple and economical, and suitable for routine regulatory inspections.

In the second method, SPME-MS yields a spectral signature characteristic of the sampled atmosphere in a few minutes. The information contained in the SPME-MS spectra was found to be closely correlated with the odour characteristics of the lipid phases.

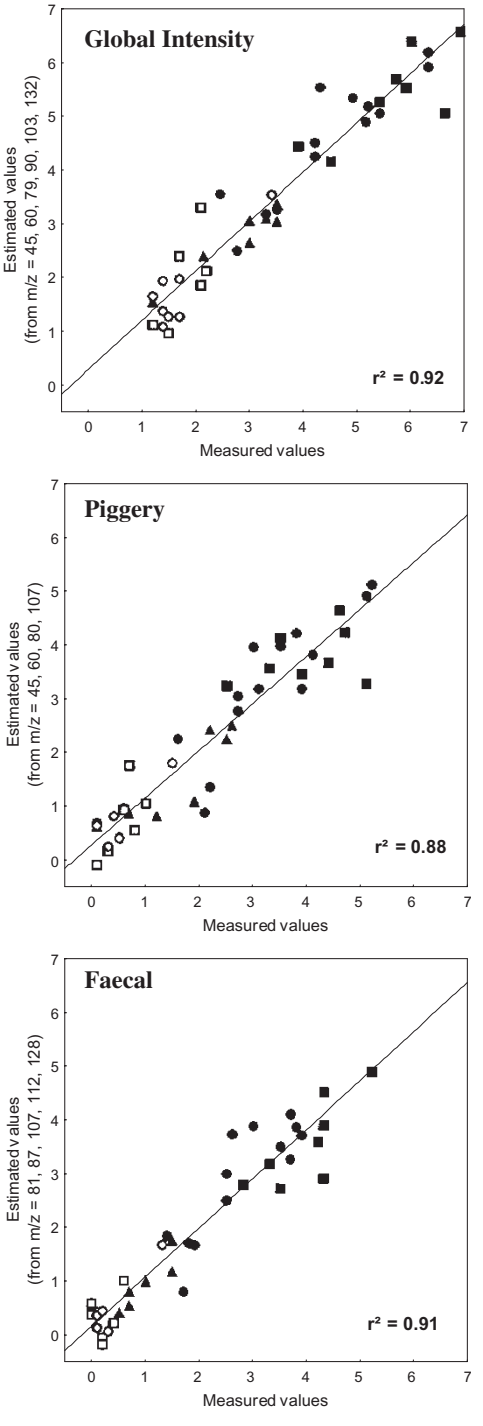

FIGURE 6 Correlations between scores estimated from SPME-MS instrument data and average scores assigned by the assessor panel for the three descriptors selected. Rearing conditions: ■ = weaned piglets on slatted flooring, □ = weaned piglets on litter, ● = fattening pigs on slatted flooring, ○ = fattening pigs on litter, ▲ = suckling sows on slatted flooring.

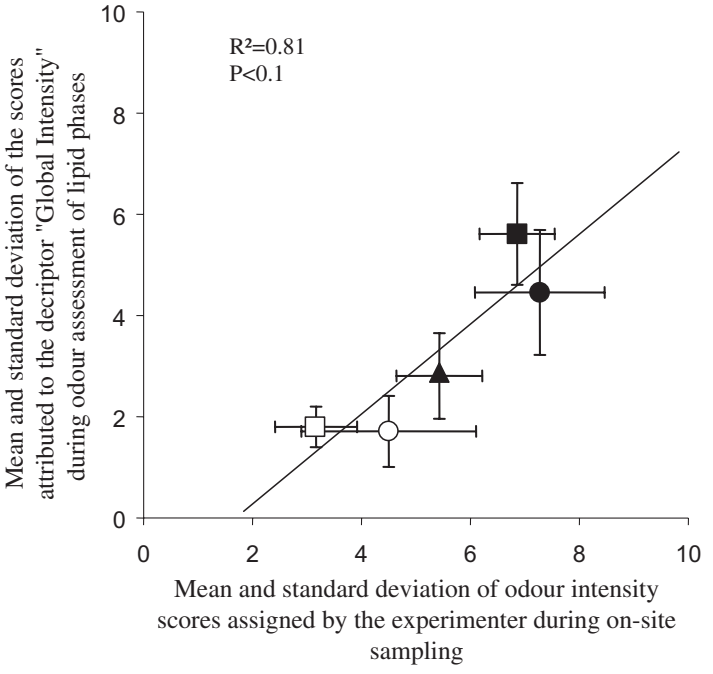

FIGURE 7 Correlations between odour intensity scores assigned by the experimenter during on-site sampling and the descriptor "Global Intensity" perceived during odour assessment of lipid phases. Each point corresponds to the mean of scores for the following five piggery sites: weaned piglets on slatted flooring (■), weaned piglets on litter (□), fattening pigs on slatted flooring, (●), fattening pigs on litter (○), suckling sows on slatted flooring (▲).

The exploitation of the data obtained by these two methods confirmed that the odour intensity is closely linked to the type of floor used. Piggeries using litter were found to generate less odour than those using slatted flooring.

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