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The development of direct headspace sampling and analysis of volatile organic compounds from broiler litter

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Abstract

As global population continue to expand, poultry production has increased to supply high quantities of chicken meat to consumers. Suburbia encroachment on rural landscapes has resulted in increased odour complaints from public. Wastes produced of livestock facilities are major cause of odour complaints received. Often assessment of odour complaint and regulating odour nuisance become great challenge due to inaccurate identification and evaluation odorants emitting of sources. In Malaysia air quality determination is often conducted based on MS 1963:2007. The aim of this study is to develop a reliable, simple, fast and cost efficient methodology to determine and characterise odorous volatiles from broiler litter material utilising headspace sampling combined with thermal desorption, gas chromatography – mass spectrometry and olfactory detection.This combined sensory/chemical analysis technique has identified 11 major odorants including reduced sulphurs, ketones, carboxylic acids, terpenes and alcohols compared to sorbent technique that identified only 4 odorants. The knowledge obtained from this assessment will assist in developing effective odour abatement and mitigation techniques to reduce odour impact to local receptors.

Keywords: odour, headspace sampling, agricultural, broiler

Introduction

Global meat chicken (broiler) production has expanded extensively to meet the growing consumers' demand. Rising demand for poultry products can be attributed to a variety of factors. As incomes rises, consumers have purchase more animal-based proteins to consume. As urban areas in Southeast Asia region grow denser in population, poultry provides consumers an affordable protein source that is more readily available compared to other meats (Miller et al. 2022).

Broiler chickens are grown on thick bedding material on the floor of mechanically ventilated tunnel sheds over 7 – 9 weeks. Though the intensive livestock practice ensures minimal nuisance generation to the surrounding condition, the facilities often become a target for odour complaints due to emerging urban infringement in the rural environment (Powers et al. 2005). Odours emit from the chicken sheds due to aerobic and anaerobic microbial activities within the litter and from the animals (Mackie et al. 1998; Lacey et al. 2004; Rappert and Muller 2005; Dunlop et al. 2016). In most cases, the odour offensive characteristics increase with the accumulation of wastes over the chicken's growth period, resulting in the local residents living near the facility reporting more experience of odour annoyance, reduced quality of life and in some cases indirect health conditions (Schiffman 1998; Modak et al. 2019; Dunlop and Atzeni 2020).

To abate odour in poultry production facilities, accurate characterisation of odours using reliable and representable techniques are essential to gain a clearer understanding of the emission nature (Schiffman 1998; Lacey et al. 2004; Powers et al. 2005; Conti et al. 2022; Guo et al. 2022) and implement odour guidelines. Similar studies have been conducted in the food, water, aroma and environmental studies using gas chromatography coupled with olfactory as this technique enables the identification of volatiles with low threshold levels and offensive qualities, which are most likely responsible for the occurrence of unpleasant odour (Hong et al. 2021; Dang et al. 2022; Kozicki 2022). It is noteworthy that this practice has limited application in the assessment of

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environmental emission from intensive livestock facilities (Rabaud et al. 2003). In Malaysia air quality determination is based on MS 1963:2007 in which uses olfactometry analysis (Department of Standard Malaysia 2007). It is notable that the determination provided information on odour concentration in odour unit (OU) only than of details on odorant (s) responsible of complaints. Thus, the objective of this study is to develop a reliable methodology to identify and characterise odorants emission of broiler litter using headspace sampling combined with thermal desorption, gas chromatography – mass spectrometry and olfactory detection (TD-GC-MS/O).

Method development

In previous work conducted on livestock and poultry industry, studies were only conducted using sensory measurement using the traditional olfactometer. To obtain reliable data combining both sensory and instrumentally measured, thermal desorption-gas chromatography-mass spectrometry- olfactometry (TD-GC-MS-O) technique is selected. This technique has been used widely in the food, wine and perfume industry to sample and analyse volatile compounds but never in the livestock area.

Materials and method

Broiler litter samples were collected from a tunnel ventilated broiler shed in Queensland, Australia. Samplings were made in a 2 m radius of each points selected. The samples were sealed in clean odour free bags before being transported for TD-GC-MS/O analysis.

Sampling of volatiles

Closed vessel direct dynamic headspace sampling was used to study volatiles from broiler litter. To ensure minimum contamination, sampling vessels utilised for direct dynamic study were screened prior to use. Approximately 100 ml of broiler litter was purged through with helium (He) gas for a minute and the volatiles were concentrated on a general purpose graphitised carbon cold trap held at –10 °C for 3.5 min at a flow rate of 50 ml/min using a dynamic headspace sampler with 2 inlets attached directly to a thermal desorption unit (TDU) (Markes Unity, Markes International, UK) (*Figure 1*). Subsequently, the cold trap was rapidly heated to 290 °C for 5 min at a rate of 20 °C/s to desorb the retrained volatiles on a gas chromatography column using a transfer line held at 140 °C.

To compare the efficacy of direct dynamic headspace sampling to the commonly used sorbent tubes, litter emissions were captured on conditioned Tenax, TA sorbent tubes. A flux chamber covering litter sample was purged with high purity nitrogen gas at a flow rate of 5 L/min during sampling of litter odour on Tenax TA sorbent tubes. Volatiles were concentrated on sorbent tube at a flow rate of 100 ml/min for 30 min by an AirChek2000 air sampling pump (SKC). Tubes containing litter volatiles

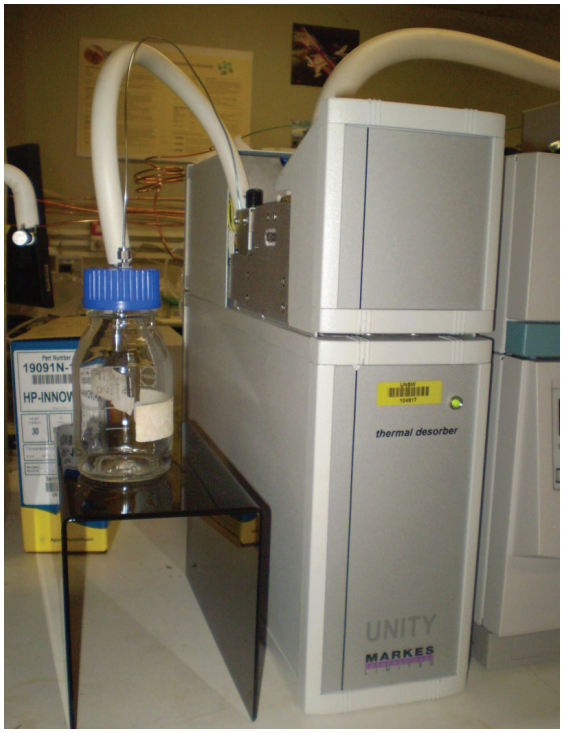

Figure 1. Direct headspace sampler showing sample vessel and thermal desorption unit (Markes International, UK)

were thermally desorbed at 275 °C for 5 min retraining volatiles on a general purpose graphitised carbon cold trap held at –10 °C in the TDU. This cold trap was later subjected to a second stage thermal desorption at 290 °C for 5 min at a rate of 20 °C/s injecting volatiles on the gas chromatography column using a transfer line held at 140 °C.

Separation and identification of volatiles

Volatiles initiated on the gas chromatography column were analysed using gas chromatography-mass spectrometry system attached to an olfactory detection port (GC-MS/O) (Agilent Technologies, USA and Gestrel, Germany) for both chemical and sensory characterisation (*Figure 2*).

Separations of volatiles were made using a polar HP-INNOWax column with dimension of 0.25 mm x 30 m x 0.25 µm (Agilent Technologies, USA), with He flowing at 1.6 ml/min. Initial oven temperature was set and held at 50 °C for 2 min, ramped at 5 °C/min to 125 °C for 10 min and finally at 10 °C/min to 200°C for 2 min. Volatiles exited the GC column were separated using a splitter at a ratio of 2:3 to a mass selective detector (MSD) (MSD 5975, Agilent Technologies, USA) and an olfactory detection port (ODP) (Gerstel, Germany). The MSD functioned at 70 eV, scanning m/z ranged from 35 to 500. Instrumental identification of separated compounds was performed by comparing the mass spectra to the NIST02 library available in the GC-MS system.

Human sensory identification was made at the olfactory detection port (ODP) (Gerstel, Germany) coupled to the GC column. This process simultaneously assesses the effluent emerging from GC. An electronic pneumatic

control module of gas chromatography maintained the flow rate to both detectors as the gas chromatography oven temperature increased during analysis run time. Two human assessors were employed to evaluate the broiler litter odorants. Both assessors were previously trained and screened using n-butanol according to AS/NZS 4323.3.2001 standard varied in sensitivity with one assessor extremely sensitive and the other averagely sensitive. However, adequate amount of training was provided to both assessors in order to reduce errors and biasness during analysis.

The ODP system consists of an olfactory port and odour input device including a headset microphone and a control pad (*Figure 3*). The odour input device assists evaluators to record their responses of intensity and qualitative characters of perceived odorants at the olfactory port using the Gerstel software. Both intensity and qualitative characters of odorous species are equally vital to confirm the most dominating compound (s) in the sample matrix. During sample analysis, the Gerstel and Agilent Chemstation softwares were set to operate simultaneously to produce aromagram and total ion chromatogram respectively. These chromatograms can be laid on each other to determine odour potent volatiles and their qualitative characters and intensities. Four levels of odorants‟ intensities as low (1), medium (2), high (3), and very high (4) have been used by assessors to quantify the offensiveness of detected odorants. On the aromagram, the heights of perceived odorants vary based on the recorded intensities.

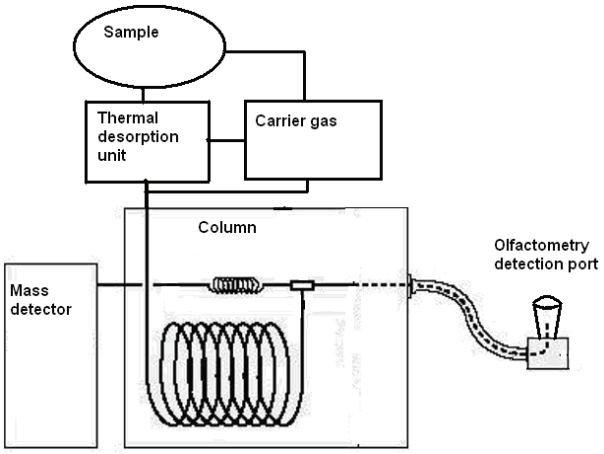

Figure 2. Schematic of TD-GC-MS-O

Results and discussion

A direct headspace sampler coupled to a TD-GC-MS/O was validated for efficacy in chemical speciation, repeatability and reproducibility to monitor variations in volatiles at ambient environment. The assessment was anticipated to provide information on the sample quantity and extraction time in order to identify the breakthrough sampling time during direct headspace sampling. Simultaneously, the validation of direct headspace technique was compared with sorbent tube extraction under identical GC conditions

Figure 3. Trained assessor evaluating the emission

using standard solutions consisting of common odorants reported in poultry facilities. As a basic requirement to determine background noise, all sorbent tubes and direct sampling vessels were screened thoroughly for contamination before use. For direct headspace extraction, volatiles were swept using purge gas onto the cold trap for 3.5 min. The pre-concentration time chosen showed sufficient extraction of the sample with post blank tests revealing high recovery of volatiles. Shorter extraction time employed on standard solution provided for poor matching of volatiles identified by the mass-spectrometry library. Meanwhile, longer sample extraction with direct headspace sampling contributed to overloading of volatiles onto the cold trap and the GC system, visible with poorly separated ‘shark fin’ shaped volatile peaks noticed on the total ion chromatogram of GC. However, the extraction time is highly subjected to changes depending on the rough estimation of sample concentration, especially for environmental based samples.

Both sampling techniques were observed to successfully discriminate all chemicals tested during validation stage at varying concentrations. From the study, the comparison of direct dynamic headspace against Tenax TA (sorbent tubes sampling determined reliable findings especially on the simplicity in the preparatory and sampling procedure including solvent free condition that was minimised to reduce interference and formation

of artefacts. Furthermore, with the developed sampling method, limited physical and chemical changes have been made to the sample matrix which enabled the sampling conditions to resemble the litter environment of a broiler shed at ambient temperature.

Numerous volatiles varying in chemical functionality were determined from broiler litter sample using direct and sorbent tube sampling techniques though samples demonstrated complex odour emissions. Major odorants obtained from litter samples were labelled on the total ion chromatograms in *Figure 4*. Fewer odorants were obtained from odour sampled on Tenax sorbent tubes compared to the direct dynamic headspace technique. *Table 1* shows volatiles collected using both techniques, exhibited large differences in relative abundance.

Direct dynamic headspace sampling analysis showed an increased sensitivity and detectability of odorants which was most likely due to volatiles being analysed as a whole headspace extract than specifically targeted compound. Sealed vessel and a short period of inert gas purge technique used further prevented continuous dilution and loss of volatiles into the atmosphere. Purging of inert gas through the litter samples enhanced volatilisation and concentration of odorants from the condensed phase to the gas phase above the sample matrix, anticipating in detection of more odorants. In contrast to this, loss and dilution of concentrations of analytes due to long period of inert gas purging above litter sample may have caused numerous odorants to be not detected or traced at low detection level using sorbent material.

Similar findings were also attained reflecting from aromagram comparing between direct headspace and sorbent tube sampling (*Figure 5*). Study also identified greater number of odorants on the aromagram than total ion chromatogram that further confirms human capability at low detection limits compared to chemical analysis via the mass selective detector. Human nose identified more odorants with higher odour intensity levels from direct dynamic headspace technique than the use of sorbent tubes (*Table 2*). However, compounds with higher relative abundance may not necessarily have an offensive character as it primarily depends on the odour characteristic and threshold limits of a compound. Moreover, it is also evident during study highly sensitive human sensory identifies greater number of odorants compared to averagely sensitive human sensory (*Figure 6*) though small concentrate of volatile released.

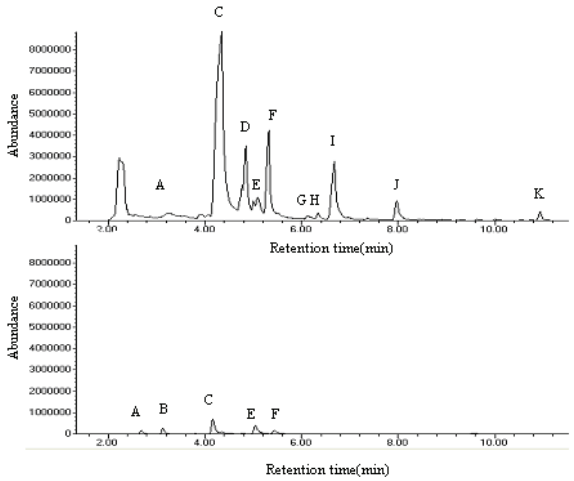

Figure 4. Total ion chromatogram for dry litter using direct headspace (top) and sorbent material sampling (below)

Table 1. Comparison of relative abundance of major odorants

Peak label	Odorant	Relative abundance with Tenax TA sorbent material	Relative abundance with direct dynamic headspace
A	Acetone	4.08E + 05	3.00E + 07
B	2-butanone	5.83E + 05	not detected
C	α pinene	1.75E + 06	1.00E + 09
D	Camphene	not detected	3.00E + 08
E	Dimethyl disulfide	1.17E + 06	1.00E + 08
F	β pinene	trace	2.00E + 08
G	α phellandrene	not detected	2.00E + 07
H	1-methyl-4-(1-methylethyl)- 1,3-cyclohexadiene	not detected	2.00E + 07
I	D-limonene	not detected	2.00E + 08
J	1-methyl-2-(1-methylethyl)- benzene	not detected	6.00E + 07
K	1-methyl-4-(1-methylethenyl)- benzene	not detected	2.00E + 07

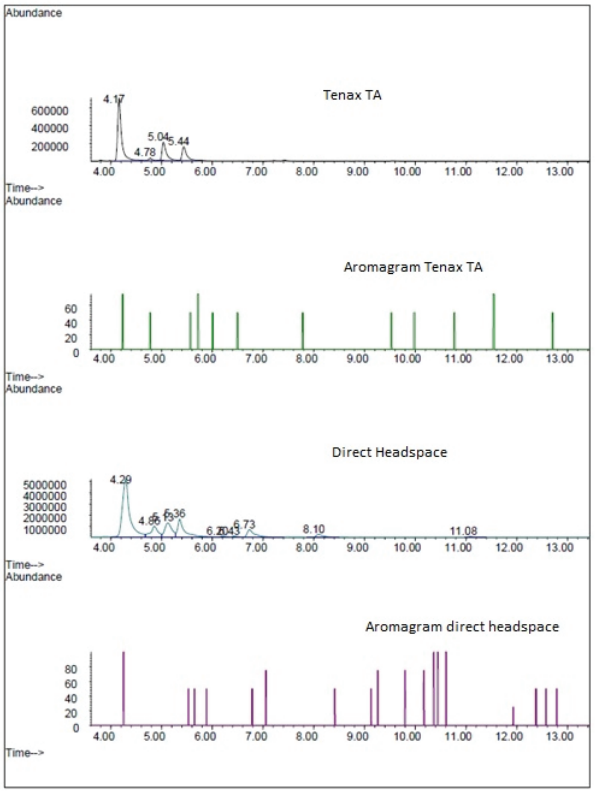

Figure 5. Comparison of odorants identified by highly sensitive human detectors based on total ion chromatogram and aromagram for both direct headspace and Tenax TA sorbent tube sampling

Table 2. Litter odorants identified by human detectors

Peak label	Highly sensitive human				Averagely sensitive human			
	O_D	I_D	O_T	I_T	O_D	I_D	O_T	I_T
A	trace	2	ash	2	none	0	none	0
B	trace	2	solvent	2	none	0	solvent	3
C	pine	3	pine	3	none	0	none	0
D	chemical	3	trace	3	none	0	none	0
E	manure		manure	3	none	0	none	0
F	resin	3	resin	3	chemical	2	none	0
G	none	0	none	0	none	0	none	0
H	foul	3	none	0	none	0	none	0
I	citrus	3	trace	3	citrus	3	none	0
J	smoke	2	trace	2	smoke	2	none	0
K	foul	2	trace	2	foul	2	none	0

O_D = odour description with direct sampling; I_D = perceived odour intensity with direct sampling; O_T = odour description with Tenax tube; I_T = perceived odour intensity with Tenax tube

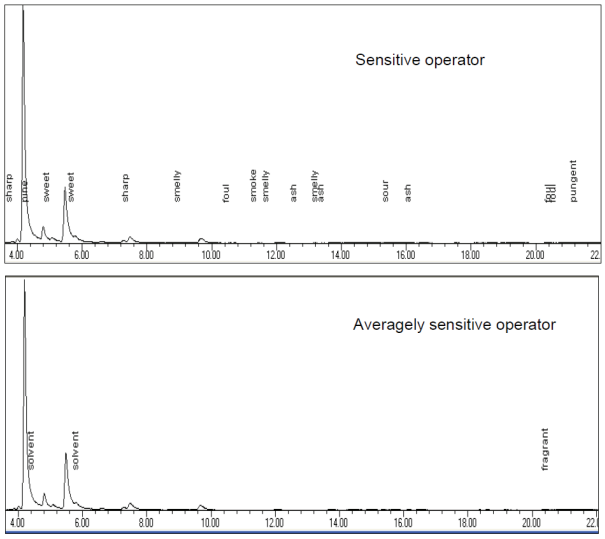

Figure 6. Comparison of odorants identified by highly and averagely sensitive human detectors based on total ion chromatogram of broiler litter sample

Conclusion

Direct dynamic headspace sampling coupled to gas chromatography-mass spectrometry/olfactory (GC-MS/O) was successfully employed to analyse odorants from broiler litter. This method has exhibited more advantages compared to sampling of volatiles using sorbent tubes. The sampling technique offered simplicity in preparation, constant repeatability and sensitivity of both human and instrumental parameters in detecting odorants in small quantity in a short analysis period. Elimination of solvent and minimal physical and chemical changes are highly notable process elimination to reduce sample and analyte degradations, interference of contaminants and the formation of artefacts. Moreover, characterisation of odorants using human and chemical detectors coupled with direct dynamic headspace sampling is anticipated to assist in selecting and implementing effective odour abatement and mitigation techniques to reduce odour impacts on local receptors.

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