A Comparative Performance Study using Dynamic Headspace Sampling and Sorbent Tube Methods of Broiler Litter Odour

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Received:- 24 February 2025/ Revised:- 05 March 2025/ Accepted:- 11 March 2025/ Published: 31-03-2025 Copyright @ 2025 International Journal of Environmental and Agriculture Research This is an Open-Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (https://creativecommons.org/licenses/by-nc/4.0) which permits unrestricted Non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abstract—Broiler production emission is often obnoxious and little is known about complete speciation and levels of volatiles from poultry production due to challenges in selecting reliable sampling technique with less amendment to volatiles' characteristic. This study aimed to compare the performance between Tenax TA sorbent tube and dynamic headspace sampling methods to distinguish chemical and olfaction analyses of odorants in broiler litter emission using standard solutions. Standard solutions containing 13 volatile compounds at 50 ppmv, 100 ppmv and 250 ppmv were sampled using Tenax TA sorbent tube and dynamic headspace sampler. The samples were analysed using thermal desorption gas chromatography-mass spectrometry olfactometry system (TD-GC-MS/O), providing data of human olfactory for identification and description of odorants detected. The Tenax TA sorbent tube and dynamic headspace sampler have successfully determined all volatiles used at all three concentrations with the Tenax TA sorbent tube sampling exhibiting relevant multiplication of peak area of volatiles corresponding to concentration of standard solution. Hence, both methods can be compared directly for qualitative information (chemical and olfactory characteristic) than quantitative information. This is evident with the strong occurrence of volatile compounds elucidating at similar retention time using Tenax TA sorbent tube and dynamic headspace sampler using all three standard solutions under identical TD-GC-MS/O analysis. Fast and simplification in sampling technique is observed using dynamic headspace sampler compared to Tenax TA sorbent tube that it would be a better direct source sampling solution.

Keywords—Broiler Litter, Odour, Sorbent Tube, Dynamic Headspace Sampling, TD-GC-MS/O.

I. INTRODUCTION

Demand for meat chicken (broiler) expands extensively with growing population and rise income globally (Miller *et al.* 2022). Broilers are usually grown on bedding material in 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 encroachment in the rural environment (Powers *et al.* 2005). Odours from broiler shed are generally generated through aerobic and anaerobic microbial activities within the litter and from the animals (Rappert and Muller 2005; Dunlop *et al.* 2016). Occurrence of odour annoyance increase with accumulation of waste sources as litter over chicken growth period. This incident results in annoyance complains from the local residents living near the facility (Schiffman 1998; Modak *et al.* 2019; Dunlop and Atzeni 2020) and also personnel's working in the facility (Marinella *et al.* 2014). To abate odour in poultry production facilities, accurate sampling and 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 2020; Guo et al 2022) and to implement odour guidelines.

Sampling of odours from a matrix at ambient condition is often critical and challenging (Laura *et al.* 2013). Odorous emissions are combination of varieties of compounds with vast range of quality and quantity (Eva *et al.* 2011; Shicheng *et al.* 2010 and Lorenzo *et al.* 2022). Techniques of volatile collection mostly include solvent extraction, sorbent tubes and solid phase micro-extraction in which involves enrichment and collection of representative volatiles, especially those with low concentration, on a suitable medium prior to analysis (Eva *et al.* 2010). However, these techniques also may cause losses of volatile organic compounds as the sorbent materials tends to absorb water in high humidity circumstances and during multiple stages of stripping and loading of odours, that may change the characteristics of its components (Noelia et al 2010). Focusing of volatiles onto sorbent materials or cold trap offers adequate approach to obtain micrograms and nanograms per litre of concentrated

volatiles, resulting in the least amendments on the gas sample and sample matrix and interference of solvent. The desired volatiles are adsorbed on a sorbent material or cold trap at a preset flow rate prior to thermal desorption. Basic prerequisites are the choice of sorbents which are dependent on the affinity of desired chemical, appropriate breakthrough volume for all volatiles present in a gaseous sample, low water affinity, high recovery of volatiles during thermal desorption, low reactivity with volatiles adsorbed and the absence of the formation of artefacts (Pillonel *et al.* 2002 and Tholl *et al.* 2006). No one sorbent material is capable of adsorbing all kinds of volatiles and most sorbent material has affinity towards a particular volatile functionality (Munoz *et al.* 2002 and Ribes et al 2007). This aspect has led to the development and usage of multi-bed sorbent tubes that enables advanced levels of volatiles' focusing (Ribes *et al.* 2007). The most common sorbent materials available commercially include Tenax TA, Chromosorb carbon molecular sieves, graphitized carbon and multi-bedded sorbent materials such as Carbograph, Carbotrap C and Carbotrap/ Carbosieve (Chuandong *et al.* 2018).

Meanwhile, dynamic headspace sampling technique is used to separate volatiles above a liquid or solid prior to instrumental analysis (Curioni and Bosset 2002). It is a simple technique to collect and analyse volatiles with the least amount of deterioration and amendment of the chemicals (Bart 2001; Tholl *et al.* 2006; d'Acampora *et al.* 2008 and Hobbs *et al.* 2004) and significantly suitable for highly humid/wet samples. Dynamic headspace sampling strips volatiles of a sample matrix in a sample vessel using a continuous flow of a gas stream under non equilibrium conditions (Bianchi *et al.* 1989). Stripped volatiles are concentrated onto a sorbent material or cold trap prior to gas chromatography analysis. Two mode of dynamic headspace sampling volatiles extraction are open stripping and closed stripping (Capelli *et al.* 2013). In open system, problems related to increases in temperature and humidity or an accumulation of damaging vapours in the headspace that are eliminated by a constant air stream. Whereas, using closed-loop stripping, volatiles, especially analytes with low concentrations, are enriched onto sorbent materials during continuous circulation of the headspace air inside closed chambers with minimum air contaminants. Minor drawback of the headspace sampling techniques is related to the cleanliness of sampling apparatus, but this can be overcome with good laboratory practices to enhance the repeatability and reproducibility of the sampling method (Tholl *et al.* 2006).

Despite availability of several sampling methods for odorant analysis, identification of suitable method serving the objective of particular experimental analysis is essential. Suitability of a sampling technique depends on many factors including environmental aspects e.g ambient temperature, sampling site, costing of analysis, skill of staff and availability of compatible instrument and etc. Moreover, the selection and execution of particular experimental techniques should produce relevant output/result for optimum solution of events. Therefore, this study aims to compare the performance of between Tenax TA sorbent tune and dynamic headspace sampling for odour analysis of broiler litter emission. The broiler litter odour was represented using standard solutions containing 13 major odorants prepared based on literature reviews (Lacey *et al.* 2004; Steven *et al.* 2010 and Jiang *et al.* 2023) The sampling techniques were coupled to thermal desorption, gas chromatography – mass spectrometry and olfactory detection (TD-GC-MS/O) system for determine volatile organic compounds with odorant properties. The TD-GC-MS/O system used provides both instrumental and human sensory evaluation of isolated odorants. The study is expected to provide information on effective and suitable sampling technique selection for fast and reliably point source broiler litter odour analysis provides clearer understanding of the emission nature. The TD-GC-MS/O technique is often used in the food, water, aroma and environmental studies to enable determination of volatiles with low threshold levels and offensive qualities responsible unpleasant odour (Kozicki 2022; Dang *et al.* 2022; Hong *et al.* 2021).

II. MATERIAL AND METHODS

Standard solutions consisting of thirteen pure odorous volatiles in broiler litter odour were prepared precisely in high performance liquid chromatography grade methanol (99%, Sigma Aldrich, Castle Hill) at 50, 100 and 250 parts per million of volume (ppmv). The solutions contained: acetone (99.5%, Sigma Aldrich, Australia), 2-butanone (99.5%, Supelco, US), 3-methyl-2-butanone (99.5%, Fluka, Europe), 2,3-butanedione (99.5%, Fluka, Europe), α-pinene (99%, Aldrich, Castle Hill), toluene (99.8%, Sigma Aldrich, US), dimethyl disulfide (99%, Aldrich, Castle Hill), 1-butanol (99%, Sigma Aldrich, US), limonene(97%, Sigma, US), 3-hydroxy-2-butanone (97%, Fluka, Europe), dimethyl trisulfide (98%, Aldrich, Castle Hill), acetic acid (99.7%, Sigma Aldrich, Castle Hill) and butyric acid (99%, Aldrich, Castle Hill).

2.1 Odour Sampling:

2.1.1 Dynamic headspace sampling:

Closed vessel dynamic dynamic headspace sampling was used to study volatiles. To ensure minimum occurrences of contamination, sampling vessels utilised for dynamic dynamic study were screened prior to use. Approximately 1 μ L of respective standard solution was placed on the base of clean sampling vessel before instantly attaching to dynamic headspace

sampler for dynamic TD-GC-MS-O analysis. This dynamic headspace sampler was developed and reported by Pillai *et al.* (2010) and Sashikala and Richard (2023). The solution 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)(Fig. 1). The cold trap was rapidly heated to 290°C approximately for 5 min at a rate of 20°C/s. The process desorbs the retrained volatiles on a gas chromatography column using a transfer line held at 140°C. The process was repeated using standard solutions of 100 and 250 ppmv concentrations, respectively.



FIGURE 1: Dynamic headspace sampler attached thermal desorption unit

2.1.2 Sorbent tube sampling:

In order to compare the application of dynamic headspace sampling method to the commonly used sorbent tubes, Tenax TA sorbent tubes (Fig. 2), packed with 2,6-diphenylene-oxide polymer resin were used. Tenax TA is a hydrophobic and weak sorbent material, which has been widely used to retrain and analyse volatiles from water, environmental air, soil, human breath, plants and commercial products. The sorbent material is packed in a stainless steel tube with a dimension of 6.35 mm (outer diameter) and 89 mm (length) to allow a surface area of 35 m²/g attracting volatiles, commonly compounds with C₇ to C₁₀. Screened Tenax TA sorbent tube was loaded with 1 μ L of 50 ppm standard solution using a calibration rig that were concurrently sealed with brass caps fitted with Teflon ferules prior to analysis. Volatile loaded tube was 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. The above process was repeated using standard solutions of 100 and 250 ppmv concentrations, respectively.



FIGURE 2: Example of sorbent tube, Tenax TA

2.2 Separation and Identification of Volatiles:

Volatiles introduced on the gas chromatography (GC) column employing dynamic headspace sampler and Tenax TA sorbent tube techniques were analysed using a gas chromatography-mass spectrometry coupled to an olfactory detection port (GC-MS/O) (Agilent Technologies, USA and Gestrel, Germany) for chemical and sensory characterisation. Separations of volatiles collected were conducted using a polar HP-INNOWax column with dimension of 0.25 mm x 30 m x 0.25 μ m (Agilent Technologies, USA) with He flowing at 1.6 ml/min. The initial oven temperature was set and held at 50°C for 2 min before being ramped at 5°C/min til 125°C for 10 min and finally at 10°C/min til 200°C for 2 min. The total run time of this program was 26.50 min. As the eluted compounds exited the GC column, a splitter separated the vapour 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 under electron impact mode at 70 eV, scanning m/z ranged from 35 to 500. Chemical identification of separated compounds was performed by comparing the mass spectra to the NIST02 library available in the GC-MS system. As no scientific instrument has the capability of interpreting perceived odorants in the way a human nose does, two screened human detectors with different sensitivities (i.e. butanol detection thresholds) were used for olfactory detection of the volatiles. The detection of volatiles were recorded the odour description and intensity of the compounds as low, mild, high and very high using scale system 1-4 on the ODP recorder software (Gerstel, Germany) (Fig. 3). The odorants were identified by matching total ion chromatogram obtained from the GC-MS with peaks obtained on the aromagram from the ODP to establish the key odorants being emitted from the litter.



FIGURE 3: Operator at the odour detection port (ODP) using headset microphone and a control pad for intensity ranking

III. RESULTS AND DISCUSSION

A dynamic headspace sampler developed by Pillai *et al.* 2010 coupled to a TD-GC-MS/O was compared with Tenax TA sorbent tube 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 extraction time in order to identify the breakthrough sampling time during dynamic headspace sampling which will be beneficial during analysis of real source of broiler litter. The validation of dynamic headspace sampler was compared with Tenax TA sorbent tube under identical GC conditions using standard solutions consisting of 13 common odorants reported in poultry facilities (Lacey *et al.* 2004; Steven *et al.* 2010 and Junyann *et al.* 2023). As a basic requirement to determine background noise, all Tenax TA sorbent tubes and dynamic headspace sampling for contamination before use. The existence of carbon dioxide at retention time 2.18 min of Tenax TA and 2.44 min of dynamic headspace sampler were obtained.

During study, dynamic headspace samples were pre-concentrate on a general graphitised carbon cold trap placed in the thermal desorption unit. The introduction of analytes onto GC column occurred was in single stage thermal desorption procedure since dynamic headspace concentrates volatiles directly onto the cold trap. This process is anticipated to reduce deformation/amendment or losses of volatiles compounds due to multiple stages of thermal desorption. Elisa *et al.* (2023) has reported some compounds, in particular aldehydes and esters of carboxylic acids compounds such as propanal, hexanal and acetic acid and methyl ester were determined using non sorbent techniques, similar to dynamic headspace sampling. In addition, detection of these compounds that are characterised with low odour threshold limits are important for olfactory analysis (Elisa *et al.* 2023). Similarly, Junyaan and co-workers have noted Tenax TA may not the best sorbent of choice for substances with medium polarity or low carbon number (Junyaan *et al.* 2023), that this characteristic may be become a hinderance during real case sampling to collect complete volatiles available in a sample matrix, hence making dynamic headspace sampler a better choice instead.

The cold trap releases the trapped volatiles into the GC system. 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 poor matching of volatiles identified by the mass-spectrometry library. Meanwhile, longer sample extraction with dynamic headspace sampling contributed to overloading of volatiles onto the cold trap and the GC system. This was 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 estimation of sample concentration, especially for environmental based samples.

Meanwhile for Tenax TA sorbent tube collection, volatiles trapped onto sorbent material were desorbed to be pre-concentrated onto the cold trap in thermal desorption unit where a second thermal desorption occurs to introduce analytes onto GC column. A temperature elevated flow path connection consisting of fused silica was used to link the thermal desorption unit and the GC-MS/O system. Increased temperature and appropriate carrier gas flow rate were required to transfer volatiles onto GC column while reducing the condensation of analytes along the transfer pathway. However, extreme precaution was practised during temperature setting at all levels during thermal desorption processes to prevent oxidation of volatiles attributed to exposure to high temperature.

Both sampling techniques were observed to be successful in discriminating all chemicals tested during validation stage at varying concentrations. Using the dynamic headspace, all volatiles were determined effectively at concentration as low as 50 ppmv except for acetone, 3-hydroxy-2-butanone and butanoic acid that required higher concentrations. Two important aspects owing to this circumstance were: a) further dilution with purge gas during sampling of volatiles onto the cold trap that reduce the concentration of substance and increase the detection limits in the GC system and b) the co-efficiency of a substance to volatilise from a condensed sample phase to the headspace gas phase that could have caused overlap of volatile separation. Meanwhile the Tenax TA sorbent tube method revealed all chemicals at concentration of 50 ppmv.

It is notable that the volume of samples carried onto the cold trap between sampling procedure differed attributed to varying sample pre-concentration technique though both techniques used 1 μ L of standard solutions. Only partial amount of 1 μ L was purged on cold trap within selected period using dynamic headspace sampler compared to Tenax TA sorbent tube that loaded complete 1 μ L of standard solution. Despite owing to inadequacy to compare both techniques directly for chemical quantitative information, the capacity of dynamic headspace sampler to determine volatiles at low volume is highly notable. The extracting techniques are comparable directly for volatiles qualitative information. The occurrence of elucidating volatiles' retention time from the GC-MS system appeared to be consistent between the sampling methods, also presenting the reliability of the GC-MS system in responding to the chemicals tested. Fig 4 shows comparatively similar occurrence of odorants at retention times between the two sampling techniques used. Adequate repeatability between extraction methods was obtained. It is evident from the spectra that both dynamic headspace sampler and Tenax TA sorbent tubes were providing consistency volatiles retention time and abundance reflecting on the reliability of sampling and analysis system respectively (Fig. 5).



FIGURE 4: Standard solution with significant similarity of retention time of volatiles Retention time (min): 4.06 =3-methyl-2-butanone, 4.57 = 2,3-butanedione, 5.22 = alpha pinene, 5.62 = toluene, 6.26 = dimethyl disulfide, 7.62 = 1-butanol, 8.86 =limonene, 11.21 = 3-hydroxy-2-butanone, 13.53 = dimethyl trisulfide

In addition, the GC-MS/O system responded efficiently in discriminating the variation in volatiles abundances (Fig. 6) and perceived odour intensity corresponding to differing standard solution concentrations. Good recovery of volatiles tested was revealed using Tenax TA sorbent tube sampling which could be due to complete thermal desorption of the sorbent tube and cold trap in thermal desorption unit. Tenax TA sorbent tube has tendency to exhibit good level of sample recovery, approximately 98% (Jacek *et al.* 2005). It is also visible from Fig. 7, which spectrums show relative multiplication of peak area of respective volatiles with increase in standard solution concentration. Unlike dynamic headspace sampler, spectrums exhibited smaller peak area compared to Tenax TA of respective standard solution concentration. Nevertheless, the dynamic headspace sampler was successful in detecting and exhibiting changes in standard solution concentration. Perhaps longer extraction time of volatiles needed to improve recovery using dynamic headspace sampler.



FIGURE 5: Good repeatability of sampling methods on volatiles' retention time and abundance obtained of 50 ppmv standard solutions



FIGURE 6: TICs of standard solutions with significant differences exhibited with increases in standard solution concentrations

Focusing on the olfactory qualitative analysis obtained using GC-MS/O, the general trends observed for olfactometric analysis are confirmed between both sampling techniques. Procedure used to determine volatiles of potential odorants is shown in Fig. 7 and Fig. 8 for both sampling techniques. The total ion chromatograms were overlaid with olfactory responses (osmegram) to match odorants according to overlapping retention times. Comparison of chromatograms show relevant utilisation of method used to study volatiles using instrumental and human sensory evaluation which is important in drafting guideline and regulations as the effort may assist in odour assessment and abatement through determination and understanding of the key odorants within poultry odour that cause odour nuisance. The cause of those odorants needs to be identified in developing better odour reduction strategies. Panelists used have successfully determined compounds used in standard solution that is evident with precise overlay of olfactory note on compounds peak accurately. Difference in heights of peaks on osmegram represents intensity of the perceived smell as some compounds sustain low threshold limit. The higher the peak on osmegram, the stronger perceived smell of the particular compound.

Table 1 shows comparison of olfactory responses for standard solution via dynamic headspace sampling and sorbent tube using identical GC-MS/O parameters. Olfactometry characteristic of most volatiles were determined by both Tenax TA and dynamic headspace sampler. However, olfactory description for acetone at all concentrations were not determined by panelist using both sampling methods. Similar case for 2-butanone, 3-methyl-2-butanone 2,3-butanedione and 3-hydroxy-2-butanone using dynamic headspace sampler at 50 ppmv concentration. The olfactory responses produced for both sampling technique provided similar description of odorants but varied in terms of intensity attributed to varying abundance of respective chemicals sampled. During increase in concentration, olfactory description was noticed to change from pleasant to annoyance. This is observed for compounds with olfactory descriptive note as 2-butanone, 3-methyl-2-butanone, α pinene and toluene with pleasant olfactory note though at higher concentration. Meanwhile 2,3-butanedione and dimethyl disulfide, dimethyl trisulfide and butanoic acid produced offensive olfactory note even at lowest concentration tested as these compounds sustain lower threshold characteristics compared to other compounds.

Although intensity increases with concentration, an increase or decrease in concentration will not produce a corresponding proportional change in odour intensity as perceived by a human subject. Some odours containing offensive olfaction note can become intense at relatively low concentrations while the pleasant odours like lemon and pine require high concentration before considered intense. An odour with a strong intensity at low concentrations may cause odour problems even at low residual levels. Intensity is how a panellist perceives the magnitude (strength) of an odour once it is above its threshold (Sashikala and Richard 2022). This incident is evident on Figure 7 and Figure 8, 2,3-butanedione and butanoic acid at retention time between 4.5-5 min and 19-20 min respectively, with small signal intensity on total ion chromatogram produced high strength response on osmegram from panellist due to low threshold limits of the compound.

Compounds	Olfactory responses					
	50 ppmv		100 ppmv		250 ppmv	
acetone	Dynamic	Tenax	Dynamic	Tenax	Dynamic	Tenax
2-butanone		solvent	solvent	solvent	solvent	solvent
3-methyl-2- butanone		solvent	solvent	solvent	chemical	sweet solvent
2,3-butanedione		rotten cabbage	rotten cabbage	rotten vegetable	rotten vegetable	rotten vegetable
α pinene	smoky solvent	pinene	pine	pine	pine	pine
toluene	solvent	solvent	pleasant chemical	solvent	solvent	solvent
dimethyl disulfide	chemical	smoky	chemical	smoky	chemical	foul
1-butanol	smoke		solvent	alcohol	foul	foul
limonene	citrus	lemonish	solvent	alcohol	foul	lemonish
3-hydroxy-2- butanone		ash	musty	ash	musty	musty
dimethyl trisulfide	putrid, ferment	putrid, ferment	putrid, ferment	putrid, ferment	putrid, ferment	putrid, ferment
acetic acid	vinegar	foul	smoke	vinegar	vinegar	foul
butanoic acid	rancid	cheesy, rancid	rancid	cheesy, rancid	rancid	cheesy

 TABLE 1

 Sensory responses of standard solutions of dynamic headspace and Tenax TA



FIGURE 7: Comparison of TIC and osmegram identifying potential volatiles with odour properties of standard solution using Tenax TA sorbent tube method with descriptions of respective volatiles noted on TIC



FIGURE 8: Comparison of TIC and osmegram identifying potential volatiles with odour characteristics of standard solution using dynamic headspace sampling with descriptions of respective volatiles noted on TIC

IV. CONCLUSION

The dynamic headspace sampler provided a simple and rapid platform to analyse volatiles during broiler litter standard solution. The main advantage of the dynamic headspace is the potential of the technique to collect volatiles with minimal sample preparation robust extraction and speciation of volatiles in original form besides reduction in the time required for volatiles collections prior to GC analysis. In addition, the capacity of dynamic headspace sampler to determined volatiles at lesser quantity used in Tenax TA sorbent tube is proven though quantitative analysis needs improvement. In comparison to Tenax TA sorbent tubes, dynamic headspace sampling able to exhibit relevant chemical and olfactory analysis using TD-GC-MS/O system at good repeatability comparatively with Tenax TA sorbent tube. In future, the dynamic headspace sampling can be qualitatively beneficial and reliable as Tenax TA sorbent tube. In fact the engagement of both sampling methods for broiler litter samplers or other live samples can provide information of diverse range of chemicals in odour samples and assist in drafting of odour regulations and guidelines.

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