



Analytical Methods

Detection of poultry spoilage markers from headspace analysis with cryoadsorption on a short alumina PLOT column[☆]Tara M. Lovestead, Thomas J. Bruno^{*}

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ABSTRACT

A rapid and simple diagnostic tool for the early detection of meat spoilage would be invaluable to ensure meat quality during production, distribution, and retail. Recently, we developed an improved purge and trap method for sampling low volatility, as well as volatile, compounds by applying low temperature collection on short alumina-coated porous layer open tubular (PLOT) columns. This method was applied to the analysis of both fresh and spoiled poultry to identify marker compounds that could be used as indicators for poultry spoilage. Samples of chicken breast were crimp-sealed in individual autosampler vials and maintained at 25 °C for either a day or 2 weeks. Two weeks were sufficient to ensure severe spoilage. The headspace was sampled by cryoadsorption for 10, 20, or 30 min; and the analytes were then separated, identified, and quantified with gas chromatography and mass spectrometry. Six potential markers for poultry spoilage were identified in the headspace of spoiled chicken: dimethyl disulphide; dimethyl trisulphide; phenyl sulphide; methyl thiolacetate; allyl methyl sulphide; and 2,4,6-trimethylpyridine. Additionally, isophorone was detected in the headspace of both the fresh and the spoiled chicken; its origin is unknown but is suspected to come from the packaging. The applicability of this method to detect chicken spoilage in a commercial setting was tested by sampling the air above spoiled chicken breast that was maintained in its original retail packaging, as obtained direct from a commercial vendor, for 2 weeks at 25 °C. Sampling was done via a modified, room-temperature approach with an activated PLOT column and a motorised pipette filler/dispenser. Five of the above compounds were also identified with this approach: dimethyl disulphide; dimethyl trisulphide; phenyl sulphide; 2,4,6-trimethylpyridine; and isophorone.

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1. Introduction

A simple diagnostic tool for rapid detection of meat spoilage prior to “off-odour” detection or visible signs of spoilage would be invaluable for food and consumer safety inspectors. One avenue for detecting meat quality and suitability for distribution is to check for the volatile organic compounds (VOCs) emitted upon lipid and/or protein oxidation (Olsen et al., 2005). Analysis of VOCs is a well established field (Boswell, 1999; Drozd, Novak, & Rijks, 1978), commonly used to test for food quality and safety. It is also important in the development of flavours and fragrances, and for monitoring air, drinking water, and soils (Drozd et al., 1978; Helmig, 1999). Little work has been done to isolate, identify, and/or quantify trace volatiles or compounds of relatively low volatility that could serve as markers for meat spoilage; however, the latter

aspect of quantification, especially with low uncertainty, is particularly important, yet extremely difficult to achieve.

One important way to investigate VOCs is the use of headspace analysis (Boswell, 1999; Ioffe, Vittenburg, & Manatov, 1984; Preto, Berenguer, Marhuenda, & Cardone, 2000; Wasik, Janicki, Wardencki, & Namiesnik, 1997; Wu & Fung, 2002). This is a technique in which a gas that has previously been in contact with a condensed solid or liquid phase is examined for the presence of volatile compounds. Headspace sampling methods can be either static or dynamic. Static methods typically involve pressurising a vial containing the sample and then sampling with either a gas-tight syringe, a multiport sampling valve, or with solid phase microextraction (SPME) (Pawliszyn, 1997; Wercinski, 1999). Dynamic methods typically involve applying a flow of carrier, or sweep, gas to the vial containing the sample and passing the sweep gas (along with any VOCs in the headspace) out of the sample matrix and vial and through either a cryostat, adsorbent, or solvent to collect the VOCs (Bruno, 2009). Headspace analysis is often referred to as the purge and trap method. After the VOCs are trapped, they are concentrated, separated, and analysed, often

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with gas chromatography (GC) (Boswell, 1999; Helmig, 1999; Ioffe et al., 1984; Namiesnik & Zygmunt, 2002; Preito et al., 2000).

Headspace analysis has been used by several research groups to investigate the VOCs emitted from meat, e.g., pork and poultry, under different packaging and storage conditions (Ahn, Nam, Du, & Jo, 2001; Olsen et al., 2005; Senter, Arnold, & Chew, 2000). This technique, however, is more difficult to apply to analytes in the headspace that are of low volatility and/or at trace quantity. Such applications of headspace analysis for compounds of low volatility or of trace quantity typically require long collection periods to obtain sufficient sample mass for analysis and identification. Most commercially available purge and trap equipment was developed for measuring VOCs over aqueous samples. As a result, these instruments typically have limited sweep times for the purge cycle because volatile compounds do not require extremely long collection times. Additionally, since samples are typically aqueous, the sample holder temperature is typically limited to less than 100 °C. These instruments are therefore difficult, if not impossible, to use to analyse compounds of low volatility, to obtain precise quantitative measurements, and for analytical studies performed as a function of collection temperatures.

We have developed an improved dynamic headspace analysis technique that makes use of cryoadsorption on short alumina-coated porous layer open tubular (PLOT) columns (Bruno, 2009). We have used alumina as the adsorbent in most of our work with this technique because it is robust and can be used with alkaline solvents; however, when the measurement warrants, we can easily use silica, porous polymers, and sol–gel phases as well. After headspace collection, the cryoadsorber column is removed and the constituents are collected, separated, and identified, typically with gas chromatography and mass spectrometry (GC–MS) (Bruno & Svoronos, 2006; NIST/EPA/NIH Mass Spectral Database).

In this paper, we present the application of cryoadsorption to the analysis of both fresh and spoiled chicken to analytically determine possible compounds, including trace compounds, to be used as indicators for chicken spoilage. The goal is to develop a rapid and facile technique for monitoring chicken spoilage during packaging, distribution, and storage. One advantage of this technique over previous methods of detecting spoilage is that trace detection is possible; therefore, spoilage could be detected well before “off-odour” is detected and visual signs of spoilage are present. Additionally, the ability to collect solutes of relatively low volatility is another advantage. Last, we report the detection of a subset of the potential marker compounds that were identified using cryoadsorption for chicken spoilage in this work in the headspace of a package of chicken breast meat that was maintained in its original retail packaging for 2 weeks at room-temperature. For this headspace collection, we used a modified sampling technique that is more akin to an approach that could be used for in-the-field detection.

2. Experimental

We obtained skinless, boneless chicken breast meat from three separate commercial vendors. Two of the three chicken packages were used to obtain chicken samples to determine potential marker compounds in the headspace above spoiled chicken. The third package was used to test the field applicability of a modified headspace collection technique to detect the potential spoilage marker compounds. One chicken breast from each of two packages was selected. Six chicken samples were cut into approximately 250–300 mg monoliths from each of these chicken breasts for a total of 12 chicken samples. These samples were crimp-sealed into individual autosampler vials and maintained at 25 °C for either 24 h or 2 weeks. Two weeks were adequate to ensure for severe spoilage.

To analyse the headspace of the spoiled and fresh chicken samples, we used cryoadsorption on short alumina-coated PLOT columns (Bruno, 2009). Figs. 1 and 2 show the experimental apparatus that was developed in our laboratory, and the PLOT column, respectively, that were used in this study. During the measurement, the vial was placed in a temperature-controlled chamber (the oven in Fig. 1). A capillary was inserted to direct a flow of the sweep gas, i.e., helium (He), into the vial, and an activated alumina-coated PLOT column (see Fig. 2) was inserted to allow the sweep gas and the constituents in the headspace to flow out of the vial. To increase the collection efficiency, the PLOT column was housed in another temperature-controlled chamber, the cryostat, which was cooled with a vortex tube (see Fig. 1). Temperatures as low as –40 °C are easily attainable with this arrangement (Bruno, 1994). In practice, it is not always necessary to use temperatures below 0 °C, which is the temperature that was utilised in this study. Sample breakthrough from the cryoadsorber capillary was checked by allowing the sweep gas exiting the PLOT column to bubble through a vial (see Fig. 1) with solvent and a small amount of a keeper (acetone with a drop of tetradecane). This solution was then analysed for compounds that did not adsorb onto the PLOT column.

The flow rate of the carrier gas, He, at the exit end of the PLOT column was monitored and the duration of headspace collection recorded. Flow rates were approximately 0.5–1 mL/min. Note that although we desire a relatively low flow rate to avoid turbulence and mechanical disturbance to the meat sample, the actual flow rate value is not very important. It is only critical that the flow rate be uniform, an aspect that has been discussed previously (Bruno, 2009). Collection periods were established for 10, 20, and 30 min. Each crimp-sealed sample of chicken was used only once. Four samples were analysed for each collection period. Analytes were removed from the PLOT column by solvent desorption with reagent grade acetone and were analysed with GC–MS (30 m capillary column of 5% phenyl–95% dimethyl polysiloxane having a thickness of 0.25 µm; temperature program hold at 33 °C for 1 min, from 33 to 150 °C at 30 °C per minute, and from 150 to 250 °C at 10 °C per minute; mass spectrometer set to scan an *m/z* range from 15 to 550 relative molecular mass units gathered in scanning mode) and a search of the NIST–EPA mass spectral database (Bruno, 2009; Bruno & Svoronos, 2006; Lide, 2004–2005; NIST/EPA/NIH Mass Spectral Database). Each sample solution was subjected to seven replicate analyses. Since we know the headspace collection temperature, the sweep gas flow rate, the mass of solute in acetone recovered from the PLOT column and the sweep time, we are able to obtain precise quantification as a function of temperature of the analytes (when analytical standards are available). Recovered mass for each analyte was determined for the eluted samples on the basis of extracted ions, sometimes called single ion monitoring or selected ion monitoring (SIM), which requires standardization (Karasek & Clement, 1988; NIST/EPA/NIH Mass Spectral Database). The pure compounds (purity greater than 97%) used for standardization were purchased from a commercial supplier. Four concentrations of each standard solution were prepared by diluting the compound of interest in acetone, and calibration was done by external standardization. Each standard solution was also subjected to seven replicate analyses. Finally, before each cryoadsorber tube was used for sampling, a blank was run by eluting the capillary with acetone and analysing the resulting solution. This step was done to ensure against cross contamination.

The source of uncertainty in evaluating the recovered mass of trace components from the headspace collection is twofold; there is uncertainty in the area quantification and in the calibration. The overall uncertainty (with a coverage factor *k* = 2), associated with the recovered mass determination for each compound was

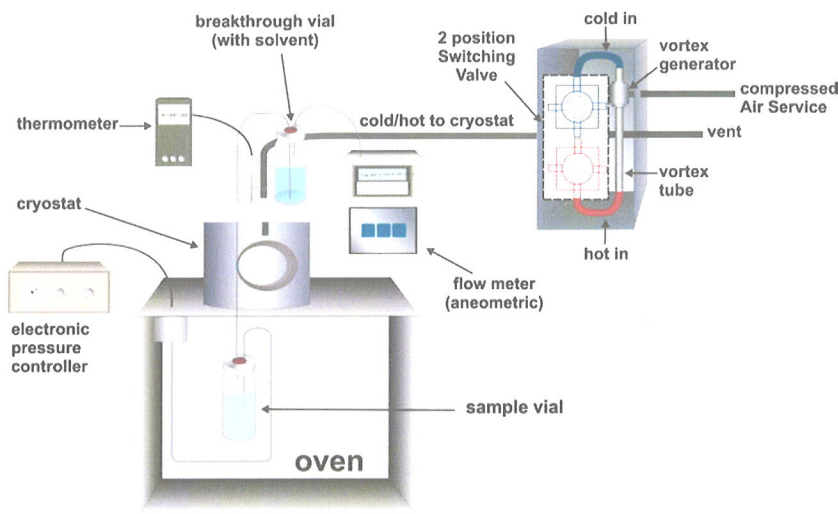


Fig. 1. A schematic of the experimental apparatus used for quantitative and trace headspace analysis with an adaptation of cryoadsorption.

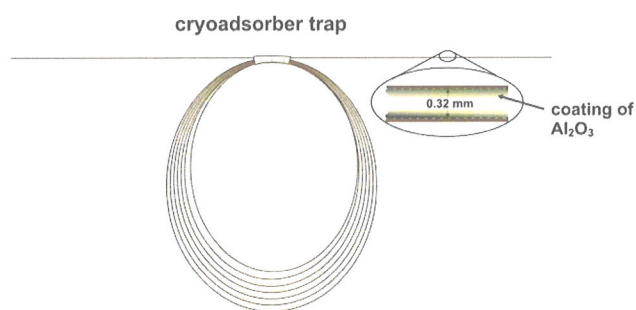


Fig. 2. A schematic of the porous layer open tubular (PLOT) column used as a cryoadsorber trap. The PLOT column is coated with aluminium oxide, is approximately 3 m long, and is coiled at a 3-in. diameter to allow the column to be housed in the cryostat chamber (see Fig. 1).

determined to be 10% at the low levels of concentration encountered in this work.

3. Results and discussion

Six potential markers for poultry spoilage (see Scheme 1) were identified in the headspace of spoiled chicken: dimethyl disulphide (CAS No. 624-92-0), dimethyl trisulphide (CAS No. 3658-80-8), phenyl sulphide (CAS No. 139-66-2), methyl thiolacetate (CAS No. 1534-08-3), allyl methyl sulphide (CAS No. 10152-76-8), and 2,4,6-trimethylpyridine (CAS No. 108-75-8). These compounds were not detected in the blanks or the headspace above fresh chicken samples. Isophorone was found in the headspace above both fresh and spoiled chicken in large amounts (CAS No. 78-59-1). Some representative properties of these compounds are provided in Scheme 1 (Linstrom; Rowley et al., 2008). Analytical standards for these compounds were used to determine the recovered mass from the PLOT column in grams per litre of He gas used during headspace collection. Dimethyl disulphide and methyl thiolacetate were standardised with ions with $m/z = 94$, 79, 61, and 45; and $m/z = 90$ and 43, respectively. Dimethyl trisulphide and allyl methyl sulphide were standardised with ions with $m/z = 126$, 79, 61, and 45; and $m/z = 88$, 73, 61, and 45, respectively. 2,4,6-Trimethylpyridine and phenyl sulphide were standardised with ions with $m/z = 121$, 106, and 79; and $m/z = 186$, 152, and 77, respectively. For convenience, these ions are also listed in Scheme 1 with each compound.

Figs. 3 and 4 show the recovered masses from 10, 20, and 30 min headspace collection, normalised by the amount of the He gas used during the headspace collection, for dimethyl disulphide (Fig. 3a); dimethyl trisulphide (Fig. 3b); phenyl sulphide (Fig. 3c); methyl thiolacetate (Fig. 3d); allyl methyl sulphide (Fig. 3e); 2,4,6-trimethylpyridine (Fig. 3f); and isophorone (Fig. 4). The data points 1–12 in these figures each represent a different chicken sample, i.e., 12 different chicken samples of approximately 250–300 mg in weight were isolated, held at room-temperature for 2 weeks and then analysed for volatiles in the headspace. The odd- and even-numbered chicken samples in Figs. 3 and 4 were cut from chicken packages one and two, respectively.

Figs. 3 and 4 reveal that there is wide variability in the range of recovered masses for each sample. This result is due most likely to the variability in sample composition, i.e., distribution of protein, fat, and connective tissue. This result is by no means a flaw in the method or study, since our goal was specifically to develop a method that required minimal sample preparation, i.e., did not require a homogenised sample. Indeed, homogenising the sample would be counter to our overarching desire to detect spoilage in the finished product, with a minimum of sample preparation. Figs. 3 and 4 also show that it is difficult to ascertain how the quantity of recovered mass correlates with the headspace collection time. Nonetheless, Figs. 3 and 4 show that a 10-minute headspace collection period was generally sufficient for detecting the compounds identified in this work. Whilst the sample-to-sample variability is high, the repeatability of the analytical method (indicated by the error bars) is excellent.

Several of the seven compounds identified in this work have been identified in both unspoiled and spoiled foods, previously by other researchers. For example, Ahn et al. (2001) investigated the impact of gamma irradiation on the volatiles produced by pork meat. They identified dimethyl disulphide and methyl thiolacetate at day 0 and day 10 in vacuum-packed, irradiated (4.5 kGy) pork samples; however, they did not find these compounds in pork that was not irradiated. They also found that volatile sulphur compounds decreased to undetectable levels when the irradiated pork samples were stored in aerobic packaging. Senter et al. (2000) have isolated dimethyl disulphide, dimethyl trisulphide, and methyl thiolacetate in the headspace above chicken samples that were stored for 5 days at 4 °C and 3 days at 13 °C, and for samples that were stored for 3 days at 4 °C followed by 1 day at 13 °C and one day at 4 °C; although, they report that these compounds did not appear consistently. Additionally, in earlier work by Freeman,

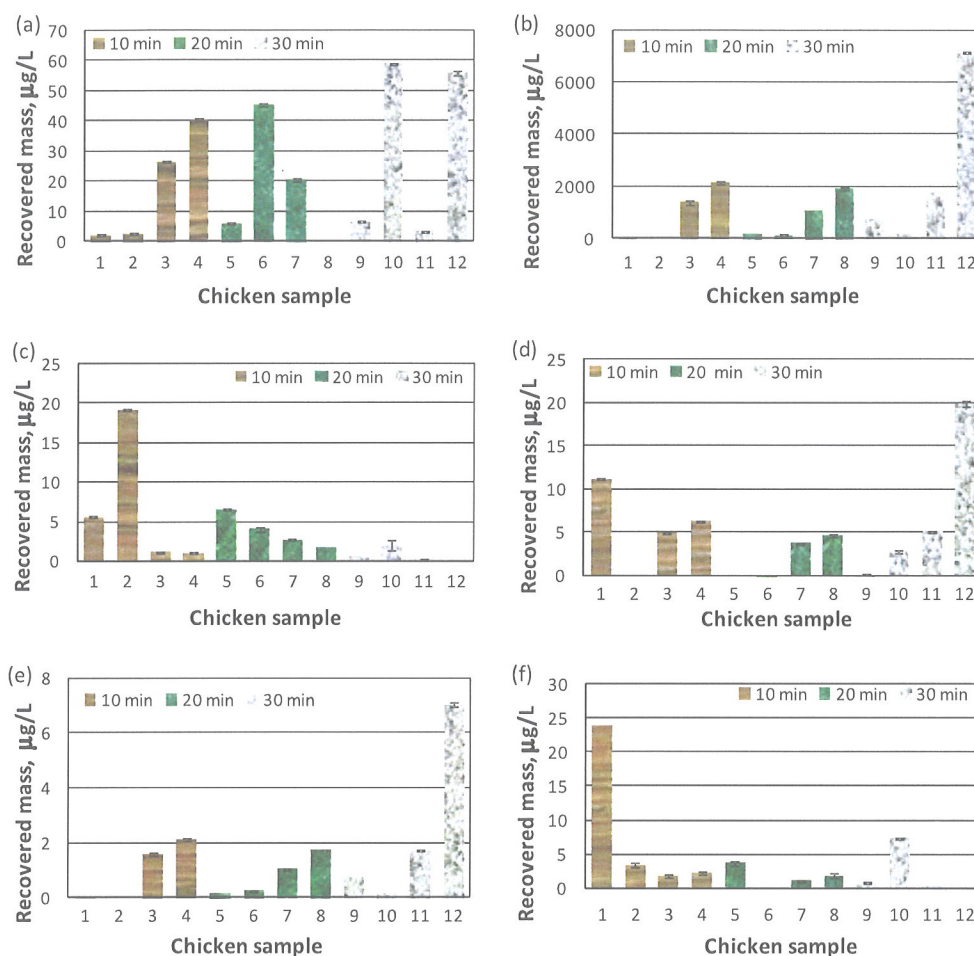


Fig. 3. Concentrations of (a) dimethyl disulphide, (b) dimethyl trisulphide, (c) phenyl sulphide, (d) methyl thiolacetate, (e) allyl methyl sulphide, and (f) 2,4,6-trimethylpyridine, expressed as recovered mass per liter of the He gas are presented for the spoiled chicken samples. Headspace collections were for 10, 20, or 30 min. Uncertainty bars indicate the uncertainty in the analytical measurement. The sample-to-sample variability is discussed in the text.

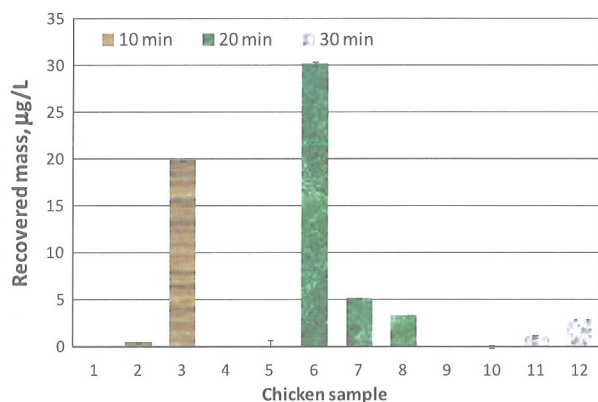


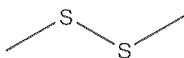
Fig. 4. Concentrations of isophorone, expressed as recovered mass per liter of the He gas are presented for the spoiled chicken samples. Headspace collections were for 10, 20, or 30 min. Uncertainty bars indicate the uncertainty in the analytical measurement. The sample-to-sample variability is discussed in the text.

Silverman, Angelini, Merritt, and Esselen (1976), methyl thiolacetate has been isolated from the volatiles produced from refrigerated chicken that has spoiled. Interestingly, this compound has also been identified in melon, strawberry, passion fruit, onions, cheese, beer, wine, whiskey, coffee, and cooked meats, and is used as a flavouring agent (IPCS INCHEM, 2001; Yannai, 2004). Allyl

methyl sulphide is a reported antioxidant found in chopped garlic and has also been identified by other groups in the headspace above Thai fried chili pastes (Amorati & Pedulli, 2008; Lawson & Gardner, 2005; Rotsachakul, Chaiseri, & Cadwallader, 2008). Thus, whilst methyl thiolacetate and allyl methyl sulphide occur naturally in some foods, these compounds, in addition to the other potential marker compounds identified, may still be indicators for chicken spoilage. A definitive conclusion regarding these markers will require additional research.

As was mentioned previously, we identified isophorone in the headspace above both spoiled and fresh chicken. Isophorone, a solvent of low vapour pressure, is used in the manufacturing of several natural and synthetic polymers, coatings, lacquers, herbicides, printing inks, and adhesives (The Dow Chemical Company, 2002). Sasaki et al. (2005) have also identified isophorone in the headspace of foods including rice, wheat, beans, miso, soy sauce, and fermented soybeans. Sasaki et al. (2005) also noted that isophorone was barely detectable in fish, meat, and vegetables. Moreover, they did not detect isophorone in the food packaging material, the suspected source for the isophorone. Isophorone has also been detected in the headspace of strawberry-tree honey samples and saffron samples (Bianchi, Careri, & Musci, 2005; de la Fuente, Sanz, Martinez-Castro, Sanz, & Ruiz-Matute, 2007; Tarantilis & Polissiou, 1997) and has been shown to be released during the thermal treatment of paprika, tomato, and marigold oleoresins, and is thought to result from carotenoid degradation (Rios, Fernandez-Garcia, Minguez-Mosquera, & Perez-Galvez,

(a) dimethyl disulfide



CAS No.: 624-92-0

InChI= 1S/C2H6S2/c1-3-4-2/h1-2H3

RMM = 94.201

 $T_{\text{boil}} = 109.75\text{ }^{\circ}\text{C}$ $T_{\text{fus}} = -84.65\text{ }^{\circ}\text{C}$

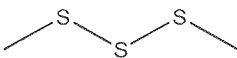
Density = 1.0627 g/mL (20 °C)

Refractive Index, $n_D^{20} = 1.52297$ (20 °C) $m/z = 94, 79, 61, \text{ and } 45$

Synonyms: 2,3-dithiabutane; methyl disulfide; (methyldithio)methane; dimethyl disulfide; dimethyl disulphide; (CH₃S)₂; UN 2381; DMDS; sulfa-hitech; sulfa-hitech 0382; (methyldisulfanyl)methane

Safety Information: Highly flammable; toxic if swallowed; harmful by inhalation; dangerous for the environment; toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment; target organs: blood and liver; stench.

(b) dimethyl trisulfide (Lide, 2004-2005)



CAS No.: 3658-80-8

InChI= 1S/C2H6S3/c1-3-5-4-2/h1-2H3

RMM = 126.267

 $T_{\text{boil}} = 41\text{ }^{\circ}\text{C}$ (0.799 kPa, 6 mmHg)

Density = 1.202 g/mL (20 °C)

Refractive Index, $n_D^{20} = 1.602$ (20 °C) $m/z = 126, 79, 61, \text{ and } 45$

Synonyms: trisulfide, dimethyl; methyl trisulfide; 2,3,4-trithiapentane; 1,3-dimethyltrisulfane; dimethyl trisulfide; dimethyl trisulphide; DMTS

Safety Information: Harmful; harmful if swallowed; irritating to eyes, respiratory system, and skin; combustible; stench.

Scheme 1. Information on components identified in the headspace of spoiled chicken. Unless otherwise indicated, boiling temperatures are at 1 atm (Linstrom; Rowley et al., 2008).

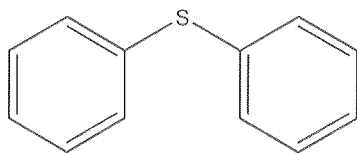
2008). The origin of isophorone in the chicken samples investigated here remains unknown. Its presence in both fresh and spoiled chicken renders it unsuitable for use as a poultry spoilage marker.

The applicability of this modified purge and trap method to detect poultry spoilage in a commercial setting was tested by sampling the air above spoiled chicken breast in its original retail packaging as obtained directly from a commercial vendor. A package of chicken breast meat was obtained and maintained unopened for 2 weeks at room-temperature. The package (349.3 g) was sampled with an activated alumina-coated PLOT column and a motorised pipette filler/dispenser under ambient conditions. One end of the PLOT column was attached to the pipette filler/dispenser and the other end was pierced through the chicken breast's

retail packaging. The pipette filler/dispenser provided the needed suction to draw the headspace into the capillary adsorber. Suction was begun only after the adsorber was inserted into the package. The headspace above the spoiled chicken breast was collected for 20 min. Following sample collection, the analytes were removed from the PLOT column by solvent desorption with acetone and analysed with GC-MS and a search of the NIST-EPA mass spectral database, as described in the experimental section and as was done for the chicken samples presented in Figs. 3 and 4 (Bruno, 2009; Bruno & Svoronos, 2006; Lide, 2004–2005; NIST/EPA/NIH Mass Spectral Database).

Table 1 shows the recovered masses for the five compounds that were identified in the spoiled chicken breast. We collected 17.7 (2.8) µg of dimethyl disulphide; 27,430 (2920) µg of dimethyl

(c) phenyl sulfide



CAS No.: 139-66-2

InChI= 1S/C12H10S/c1-3-7-11(8-4-1)13-12-9-5-2-6-10-12/h1-10H

RMM = 186.274

 $T_{\text{boil}} = 296.05\text{ }^{\circ}\text{C}$ $T_{\text{fus}} = 135.85\text{ }^{\circ}\text{C}$

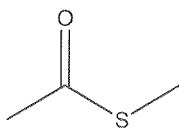
Density = 1.113 g/mL (20 °C)

Refractive Index, $n_D^{20} = 1.633$ (20 °C) $m/z = 186, 152, \text{ and } 77$

Synonyms: benzene, 1,1'-thiobis-; diphenyl sulfide; diphenyl monosulfide; diphenyl sulphide; diphenyl thioether; diphenylmercaptan; phenylthiobenzene; diphenylthiamethane; sulfide, diphenyl; 1,1'-thiobis(benzene); (phenylsulfanyl)benzene

Safety Information: Harmful if inhaled or swallowed; irritating to skin; dangerous for the environment; very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment; stench.

(d) methyl thiolacetate



CAS No.: 1534-08-3

InChI = 1S/C3H6OS/c1-3(4)5-2/h1-2H3

RMM = 90.145

 $T_{\text{boil}} = 98.15\text{ }^{\circ}\text{C}$ $m/z = 90 \text{ and } 43$

Synonyms: acetic acid, thio-, s-methyl ester; s-methyl thioacetate; methylthioacetate; s-methyl ethanethioate; ethanethioic acid, methyl ester; methyl ethanethioate

Safety Information: Highly flammable; irritating to the eyes, respiratory system, skin, and mucous membranes.

Scheme 1 (continued)

trisulphide; 3.31 (0.51) μg of phenyl sulphide; 41.2 (0.41) μg of 2,4,6-trimethylpyridine; and 22.8 (0.55) μg of isophorone in 20 min. Shorter collection periods are indeed possible, as are shorter incubation periods; however, this present work is illustrative of the concept and is a very successful first attempt to develop a room-temperature, in-the-field, poultry spoilage detection technique. Thus, not only have potential markers for chicken spoilage been identified with cryoadsorption on short activated PLOT columns, but have also detected a subset of these markers in the headspace above chicken breast meat in its original retail packaging.

4. Conclusions

An improved purge and trap method for sampling low volatility, as well as volatile, compounds, by applying low collection temperatures on short alumina-coated PLOT columns, was applied to the analysis of both fresh and spoiled chicken. Gas chromatography with mass spectrometric detection was used to separate, identify, and quantify the collected analytes. Six compounds: dimethyl disulphide, dimethyl trisulphide, phenyl sulphide, methyl thiolacetate, allyl methyl sulphide, and 2,4,6-trimethylpyridine were detected in the headspace of the spoiled

(e) allyl methyl sulfide



CAS No.: 10152-76-8

InChI= 1S/C4H8S/c1-3-4-5-2/h3H,1,4H2,2H3

RMM = 88.172

 $T_{\text{boil}} = 92.05\text{ }^{\circ}\text{C}$

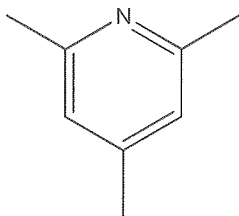
Density = 0.803 g/mL (20 °C)

Refractive Index, $n_D^{20} = 1.4714$ (20 °C) $m/z = 88, 73, 61, \text{ and } 45$

Synonyms: 1-propene, 3-(methylthio)-; allyl methyl sulfide; methyl allyl sulfide; 3-(methylthio)propene; $\text{CH}_3\text{SCH}_2\text{CH}=\text{CH}_2$; 3-(methylsulfanyl)-1-propene; 3-(methylthio)-1-propene; methyl 2-propenyl sulfide; methylallyl sulphide

Safety Information: Highly flammable, keep away from source of ignition; may be harmful by inhalation, ingestion, or skin absorption; may cause irritation.

(f) 2,4,6-trimethylpyridine



CAS No.: 108-75-8

InChI= 1S/C8H11N/c1-6-4-7(2)9-8(3)5-6/h4-5H,1-3H3

RMM = 121.180

 $T_{\text{boil}} = 170.85\text{ }^{\circ}\text{C}$ $T_{\text{fus}} = -44.2\text{ }^{\circ}\text{C}$

Density = 1.05848 g/mL (20 °C)

Refractive Index, $n_D^{20} = 1.4959$ (20 °C) $m/z = 121, 106, \text{ and } 79$

Synonyms: α,γ,α' -collidine; γ -collidine; s-collidine; 2,4,6-collidine; pyridine, 2,4,6-trimethyl; sym-collidine; 2,4,6-kollidin; a,g,a'-collidine; g-collidine; collidine

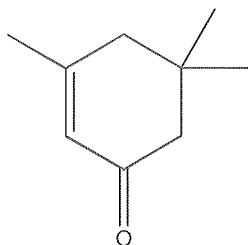
Scheme 1 (continued)

chicken. These compounds were not detected in the headspace of the fresh chicken samples, and thus could serve as markers (or trace indicators) for chicken spoilage. Isophorone was identified in the headspace of both the fresh and spoiled chicken samples. Whilst its origin remains unknown, it is suspected to come from the food packaging or handling. Cryoadsorption affords a facile approach that is well suited to the identification and quantification of trace compounds of relatively low volatility in the headspace of spoiled meat and could potentially be applied to monitor and detect spoilage in chicken well before "off-odour" is detectable. Additionally, preliminary testing with a modified

sampling approach more amenable to room-temperature collection has shown that five of the above compounds (dimethyl disulphide; dimethyl trisulphide; phenyl sulphide; 2,4,6-trimethylpyridine; and isophorone) were identified in the air above spoiled chicken breast that was maintained in its original retail packaging at 25 °C for 2 weeks. More work is needed to optimise this field sampling technique; however, we believe our initial results are very promising, as most of the potential spoilage markers identified by our laboratory testing method were observed. Extensions of this work could include investigation of the detection of pesticide residues.

Safety Information: Combustible Liquid; toxic by ingestion; toxic by skin absorption; may cause eye irritation; may be harmful if inhaled; may cause respiratory tract irritation; toxic if absorbed through skin; may cause skin irritation.

(g) isophorone (June 2002)



CAS No.: 78-59-1

InChI=1/C9H14O/c1-7-4-8(10)6-9(2,3)5-7/h4H,5-6H2,1-3H3

RMM = 138.207

$T_{\text{boil}} = 213.7\text{ }^{\circ}\text{C}$

$T_{\text{fus}} = -7.95\text{ }^{\circ}\text{C}$

Density = 0.9196 g/mL (20 °C)

Refractive Index, $n_D^{20} = 1.478$ (20 °C)

$m/z = 82, 95, \text{ and } 138$

Synonyms: isoacetophorone; isoforon; isoforone (Italian); isophoron; isophorone (ACGIH:OSHA); izoforon (Polish); NCI-C55618; 1,1,3-trimethyl-3-cyclohexene-5-one; 3,3,5-trimethyl-5-cyclohexen-1-one; 3,5,5-trimethyl-2-cyclohexene-1-one; 3,5,5-trimethyl-2-cyclohexen-1-on (German, Dutch); 3,5,5-trimethylcyclohex-2-ene-1-one; 3,5,5-trimetil-2-cicloesen-1-one (Italian)

Safety Information: Harmful in contact with skin and if swallowed; irritating to respiratory system; risk of serious damage to eyes; possible carcinogen (US); target organs: lungs, liver, and kidneys.

Scheme 1 (continued)

Table 1

Recovered mass of five potential marker compounds identified in the air above spoiled chicken breast that was maintained at 25 °C in the original retail packaging from the commercial vendor for 2 weeks. Isophorone is in italics because it was also identified in the headspace above fresh chicken. The uncertainties are in parentheses and are discussed in the text.

Name	Recovered mass (μg)
Dimethyl disulphide	17.7 (2.8)
Dimethyl trisulphide	27.430 (2920)
Phenyl sulphide	3.31 (0.51)
2,4,6-Trimethylpyridine	41.2 (0.41)
<i>Isophorone</i>	228.3 (5.46)

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