Arachidonic acid and fatty acid profiles as indicators of contamination from the leachates of animal carcasses

Hyun-Hee Lim, Ho-Sang Shin, Tae-Wan Jeon, Sun-Kyoung Shin and Young-Woo Jeung

ABSTRACT

Because many livestock with avian influenza and foot and mouth disease have been disposed of at landfills, underground water contamination is a big problem for people living near these landfills as well as burial sites. Biological oxygen demand (BOD), NH₄, total dissolved solids (TDS), chloride and potassium have been used as markers of contamination from the leachates of animal carcasses. However, livestock manure and vegetable oils from compost are also a source of these markers. In this study, we propose arachidonic acid (C20:4) as a specific indicator of leachates from animal carcasses, and the presence of arachidonic acid in underground water is suggested to be a potential marker of contamination from the leachates of animal carcasses. Based on the specific fatty acid composition, the fatty acid profiles of the carcass leachates, livestock manure and vegetable oil were examined, and multidimensional scaling (MDS) analyses of the fatty acids showed pronounced differences between the carcass leachates, livestock manure and vegetable oils in this study. MDS analyses of fatty acids can also be used as a potential indicator of contamination from the leachates of animal carcasses in groundwater.

Key words | arachidonic acid, carcass leachates, fatty acid profile, gas chromatography–mass spectrometry, indicator

INTRODUCTION

Avian influenza refers to the disease caused by infection with avian (bird) influenza (flu) Type A viruses. These viruses occur naturally among wild aquatic birds worldwide and can infect domestic poultry and other bird and animal species. Additionally, foot and mouth disease (Aphthae epizooticae) is an infectious and sometimes fatal viral disease affecting cloven-hoofed animals such as cattle, sheep, pigs, goats and deer (UKPHLS 2001). These diseases are a natural disaster for farmers and the country, because they are highly infectious and can rapidly spread from infected animals to uninfected animals through the air and by contact with contaminated farming equipment, vehicles, clothing and feed. This of course results in enormous financial damage to individuals, the agricultural industry and the country (UKEA 2001; Thompson et al. 2002).

The suspected infected animals must be isolated and not moved, and farmers must prevent contact with other livestock. The control of these diseases requires considerable effort including vaccination, quarantine, movement control and infrequently the elimination of millions of animals. The removal of infected animals and those suspected of infection is essential to overcome these diseases. The carcasses and contaminated products should always be buried or burnt. They may more often be disposed of at landfills, which should have suitable leachate and gas collection and monitoring systems. In the case of landfills, the groundwater and surface water nearby should be continually monitored to reduce the risk of undetected leaks and to promptly undertake any remedial action if necessary. Biological oxygen demand (BOD), ammonia and suspended solids as well as chloride and potassium have been measured in surface waters, groundwaters and
leachates. Ammonia and potassium have been used as key markers of pollution (UKPHLS 2003). They not only originate from the leachates of animal carcasses, but also from livestock manure and vegetable oils from nearby compost. Because it is difficult to differentiate the source using the detected chemicals, new specific markers for contamination from the leachates of animal carcasses are necessary.

Arachidonic acid is a carboxylic acid with a 20-carbon chain and four cis-double bonds; the first double bond is located at the sixth carbon from the omega end shown in Figure 1. The compound is present in phospholipids (especially phosphatidylethanolamine, phosphatidylcholine, and phosphatidylinositides) found in eukaryotic cell membranes, and is abundant in the brain, muscles and liver of animals (Crawford & Sinclair 1971; Nelson et al. 1997; Trappe et al. 2001). Because little or no arachidonic acid is found in common plants, the compound is expected to be a specific indicator of contamination from the leachates of animal carcasses.

The profiles of fatty acids have been used as a good classification method to discriminate between different sources (Kimpe et al. 2001; Aldai et al. 2006; van Ruth et al. 2010). Fatty acid profiles could also be a good indicator of carcass leachates as well as to be used to investigate any effects from the burial of animal carcasses.

To date, many analytical methods for the determination of fatty acids have been described. Among the methods, high-performance liquid chromatography (HPLC) (Raza et al. 2009), liquid chromatography–mass spectrometry (Ayorinde et al. 2000; Haefliger & Sulzer 2007; Raza et al. 2009; Wiesman & Chapagain 2009; Spitsmeister et al. 2010; Lísa et al. 2011), gas chromatography (Hajimahmoodi et al. 2005; Barros et al. 2007; Christian et al. 2007; Juanéda et al. 2007; Charef et al. 2008; Juárez et al. 2008; König et al. 2008; Yılmaz & Gecgel 2009; Hoffmann et al. 2010; Petrović et al. 2010; Sánchez et al. 2010; Wei et al. 2013), and gas chromatography–mass spectrometry (GC-MS) (Kulig et al. 2006; Griesbach et al. 2008; Thurnhofer et al. 2008; Roehsig et al. 2010; Silva et al. 2010; Guo et al. 2012) are generally used in the determination of fatty acids. Due to a low vapor pressure and bad chromatographic properties, gas chromatographic separations of fatty acids can be performed only after derivatization of the native compounds to less polar species. Fatty acids are mainly converted into their corresponding methyl esters prior to chromatographic analysis.

A method for determining the fatty acid content in meat for animal fat analysis (Carrapico et al. 2000) from the original one-step method was reported by Sukhija and Palmquist (Sukhija & Palmquist 1988). Additionally, fatty acid patterns of animals were proposed as markers of food sources in the field (Haubert et al. 2004, 2006; Chamberlain et al. 2005; Ruess et al. 2005).

In this study, the previous GC-MS methods were modified to achieve optimum hydrolysis time and the concentration of alkali solution for samples of carcass leachates, livestock manure, and vegetable oils. Additionally arachidonic acid and fatty acid profiles were tested as specific indicators of contamination from the leachates of animal carcasses.

**MATERIALS AND METHODS**

**Chemicals and reagents**

Methanol, acetone, ethyl acetate and hexane (HPLC grade) were purchased from Sigma–Aldrich (St Louis, MO, USA). Water was purified in milli-Q (Millipore Corp., Milford, MA). Myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), arachidic acid (C20:0), arachidonic acid (C20:4), and heneicosanoic acid (C21:0) as internal standard was reported by Sukhija and Palmquist (Sukhija & Palmquist 1988). Additionally, fatty acid patterns of animals were proposed as markers of food sources in the field (Haubert et al. 2004, 2006; Chamberlain et al. 2005; Ruess et al. 2005).

**Carcass burial pits and sampling**

Five pilot sites of carcass burial pits were constructed at the National Institute of Environmental Research in Inchon, Republic Korea. The surface area of each disposal pit was approximately 9 m² (3.5 m by 2.5 m), and the depth of the pit was 2.2 m. The pits were lined with a 20 mm PVC liner, and a 50 L reservoir was placed beneath the liner and connected to a well made with a perforated polyethylene pipe for leachate collection. The bottom piper was covered with 0.2–2 cm structural sand and connected to...
the leachate collection system with a peristaltic pump. Two beef cattle carcasses (approximately 700 kg) were placed in each of the four replicate pits, and 10 swine carcasses (approximately 850 kg) were placed in one pit. The five pits were backfilled with native soil and compacted with a tapping machine right after the carcasses were placed inside the pits. All the carcasses were obtained from beef cattle and swine younger than 30 months of age. Carcasses were placed in the pits 1 or 2 days after death.

A total of 76 samples (the average volume of 0.75 L) were taken monthly from the leachates of the animal carcasses from the second month to the eighteenth month after burial, respectively. Swine flesh, Korean beef cattle flesh and vegetable oils were purchased from the market in Seoul. All samples were immediately frozen and stored at −20°C until analysis.

Samples of swine and Korean beef cattle flesh of 20 g each were crushed in a homogenizer with 20 mL of hexane for 10 min. The mixture was filtered, and the hexane was evaporated under a mild stream of nitrogen to yield pure animal fat, which was used in the alkali hydrolysis, extraction and derivatization procedures.

**Alkali hydrolysis, extraction and derivatization procedure**

A total of 1 mL of 12.0 M KOH and 50 μL of heneicosanoic acid were added to 5 mL of the livestock manure and carcass leachate samples. A 5.0 mL volume of 2.0 M KOH and 50 μL of heneicosanoic acid as an internal standard solution (100.0 mg/L in acetone) were added to 0.1 g of extracted meat fat or vegetable oil samples. The sample solutions were homogenized in a homogenizer (PowerGen 125, Fisher Scientific, USA) and sonicated in an ultrasonic bath (Branson 5210, Branson Ultrasonic Cleaner, USA) for 10 min to increase the extraction yield. The solution was hydrolyzed by heating for 6 h at 100°C. The solution was extracted twice with 5 mL of hexane, and the organic layer was concentrated to 100 μL. A 0.2 mL solution of 0.1 M HCl in methanol was added to the organic layer, and the tubes were heated for 20 min at 80°C. The solution was extracted twice with 2 mL of ethyl acetate, and the organic layer was concentrated to 100 μL. A 2 μL sample of the solution was injected into the GC system.

Spiked samples were prepared by fortifying water samples (5.0 mL) at a concentration of 0.1–1000.0 mg/L and at a concentration of 100.0 mg/L as internal standard.

**Gas chromatography–mass spectrometry**

An agilent 7890 A gas chromatograph was used with a split/splitless injector (Agilent Technologies, Santa Clara, CA, USA). The analytical column was a 30 m HP-5 MS column (cross-linked 5% phenylmethylsilicon, 0.2 mm I.D. × 0.25 μm F.T). The oven temperature program was as follows: started at 100°C, held for 1 min, raised to 320°C at 20°C/min and then held for 5 min. All mass spectra were obtained with an Agilent 5975 B instrument. The ion source was operated in the electron ionization mode (EI; 70 eV, 230°C). Full-scan mass spectra (m/z 50–800) were recorded to identify the analytes at a high concentration. Confirmation of trace chemicals was done with three MS characteristic ions, and the ratio of the three MS characteristic ions and the GC-retention time were matched to the known standard compound. The ions selected in this study are described in Table 1.

**Calibration and quantification**

Calibration curves for the fatty acids were established by saponification and extraction after adding 0, 0.02, 0.05, 0.1, 0.2, 0.5, 1, 5, 10 and 20 μg of standards and 5 μg of the internal standard to 0.1 g of meat or to 5.0 mL of the carcass leachate. Heneicosanoic acid was used as the internal standard.

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**Table 1** The mass fragmentation of fatty acid methyl esters by electron ionization

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Quantitative ion (m/z)</th>
<th>Characteristic ion (m/z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl myristate (C14:0)</td>
<td>242</td>
<td>74, 87, 143</td>
</tr>
<tr>
<td>Methyl palmitate (C16:0)</td>
<td>270</td>
<td>74, 87, 227</td>
</tr>
<tr>
<td>Methyl palmitoleate (C16:1)</td>
<td>236</td>
<td>55, 69, 194</td>
</tr>
<tr>
<td>Methyl stearate (C18:0)</td>
<td>298</td>
<td>74, 87, 255</td>
</tr>
<tr>
<td>Methyl oleate (C18:1)</td>
<td>264</td>
<td>55, 69, 222</td>
</tr>
<tr>
<td>Methyl linoleate (C18:2)</td>
<td>294</td>
<td>67, 81, 263</td>
</tr>
<tr>
<td>Methyl arachidate (C20:0)</td>
<td>326</td>
<td>74, 87, 283</td>
</tr>
<tr>
<td>Methyl arachidonate (C20:4)</td>
<td>150</td>
<td>79, 91, 105</td>
</tr>
<tr>
<td>Methyl heneicosanoate (C21:0)</td>
<td>340</td>
<td>74, 87, 297</td>
</tr>
</tbody>
</table>
ratios of the peak area of standard to that of internal standard were used in the quantification of the target compounds.

**Multivariate statistics**

Multidimensional scaling (MDS) is a statistical technique for visual representation of the pattern of proximities among multidimensional systems on a visual low dimensional grid (2D or 3D). The similarity between the samples decreases with the distance in the MDS plot. In the first step, the groups for MDS were divided into individual fatty acids as follows: group 1 = myristic acid (C14:0); group 2 = palmitic acid (C16:0); group 3 = palmitoleic acid (C16:1); group 4 = stearic acid (C18:0); group 5 = oleic acid (C18:1); group 6 = linoleic acid (C18:2); group 7 = arachidic acid (C20:0), and group 8 = arachidonic acid (C20:4). In the second step, the fatty acids were divided into four groups according to carbon numbers as follows: group 1 = myristic acid (C14:0); group 2 = palmitic acid (C16:0) and palmitoleic acid (C16:1); group 3 = stearic acid (C18:0), oleic acid (C18:1) and linoleic acid (C18:2), and group 4 = arachidic acid (C20:0) and arachidonic acid (C20:4). All these statistical techniques were performed using the PASW statistics 18.0 (Chicago, Illinois, USA).

**RESULTS AND DISCUSSION**

**Fatty acid analysis**

Methods for the quantitative and qualitative analysis of fatty acids from samples of carcass leachates, livestock manure and vegetable oils were tested. An alkali hydrolysis method was selected for the hydrolysis of lipids. Lipids extracted from the swine flesh were used to determine the optimal conditions for alkali hydrolysis to fatty acids. The hydrolysis rate of fatty acids was investigated according to the reaction temperature (80, 90 and 100 °C), reaction time (1, 2, 4, 6 and 8 h), and concentration of the KOH solution (1, 2 and 3 M). When those conditions were evaluated by comparing the peak areas of the fatty acid derivatives, the optimal reaction time and temperature were 6 h and 100 °C (Figure 2), and the yield declined rapidly beyond 6 h. The hydrolysis reaction at a temperature lower than 100 °C and at a molarity lower than 2.0 M KOH solution was not completed even in 6 h. Moreover, fatty acid hydrolysis with a reaction temperature over 100 °C using a KOH solution with a molarity higher than 3.0 M was not reproducible and had low recovery. The optimal hydrolysis conditions were heating in 2.0 M KOH solution for 6 h at 100 °C (Figure 2).

After the hydrolysis, the samples were shaken for 10 minutes after adding 50 μL of 100 mg/L heneicosanoic acid and 5 mL of hexane. The distributed solvent layer was concentrated to 100 μL by nitrogen and the concentrated solution was used for the methylation step.

Optimization was performed for the methylation of the fatty acids from the samples. The reactivity of the fatty acids was tested with the detection of the corresponding fatty acid methyl ester in acidic methanol conditions. The maximum reaction yields were obtained by a reaction in 0.2 mL of 0.1 M HCl-MeOH for 20 min at 80 °C. The methyl ester derivatives were quantitatively extracted into a 1 mL of ethyl acetate, and the solvent layer was dried to 0.1 mL, and 2.0 μL of the extract were

![Figure 2](https://iwaponline.com/ws/article-pdf/16/5/1287/411415/ws016051287.pdf) | Effect of KOH molarity (a) and reaction time (b) in the hydrolysis of lipids in animal fats to fatty acids (this experiment was performed at temperature of 100 °C).
Figure 3 | Chromatograms of the control sample (a) spiked with fatty acid standards and real carcass leachates (b). The retention times of the fatty acids were 17.21 min for methyl myristate, 19.16 min for methyl palmitoleate, 19.33 min for methyl palmitate, 21.02 min for methyl linoleate, 21.09 min for methyl oleate, 21.15 min for methyl stearate, 22.55 min for methyl arachidonate, 23.01 min for methyl arachidate and 23.89 min for methyl heneicosanoate (ISTD).

Table 2 | Calibration curves, detection limits, and precision and accuracy of fatty acids

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Calibration curve (r²)</th>
<th>Detection limit (mg/L)</th>
<th>Precision and accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Linear equation</td>
<td>LOD</td>
<td>LOQ</td>
</tr>
<tr>
<td>Methyl myristate (C14:0)</td>
<td>$y = 0.1443x + 0.0367$ (0.9967)</td>
<td>0.2</td>
<td>0.0008</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>1.03 ± 0.06</td>
</tr>
<tr>
<td>Methyl palmitate (C16:0)</td>
<td>$y = 0.1581x + 0.0578$ (0.9987)</td>
<td>0.2</td>
<td>0.0013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>1.08 ± 0.04</td>
</tr>
<tr>
<td>Methyl palmitoleate (C16:1)</td>
<td>$y = 0.1051x + 0.0256$ (0.9986)</td>
<td>0.2</td>
<td>0.0007</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>1.03 ± 0.07</td>
</tr>
<tr>
<td>Methyl stearate (C18:0)</td>
<td>$y = 0.2005x + 0.0655$ (0.9979)</td>
<td>0.2</td>
<td>0.0012</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>0.97 ± 0.06</td>
</tr>
<tr>
<td>Methyl oleate (C18:1)</td>
<td>$y = 0.1053x + 0.0318$ (0.9977)</td>
<td>0.2</td>
<td>0.0022</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>0.91 ± 0.06</td>
</tr>
<tr>
<td>Methyl linoleate (C18:2)</td>
<td>$y = 0.0635x + 0.0169$ (0.9976)</td>
<td>0.2</td>
<td>0.0006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>1.03 ± 0.07</td>
</tr>
<tr>
<td>Methyl arachidate (C20:0)</td>
<td>$y = 0.2203x + 0.0430$ (0.9966)</td>
<td>0.2</td>
<td>0.0035</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>1.01 ± 0.05</td>
</tr>
<tr>
<td>Methyl arachidonate (C20:4)</td>
<td>$y = 0.0745x + 0.0270$ (0.9964)</td>
<td>0.2</td>
<td>0.0015</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>0.96 ± 0.05</td>
</tr>
</tbody>
</table>
injected into the GC-MS. No interfering peaks were observed. The derivative preparation can be performed within 20 min. The derivatives were stable under normal laboratory conditions.

The optimum derivatization conditions were applied to the analysis of fatty acids in the fortified and real samples. Figure 3 shows a GC-MS chromatogram after the acidic methylation of fatty acids in the fortified and real samples. For the GC separation of the derivatives, the use of a nonpolar stationary phase was found to be efficient. The methyl derivatives of fatty acids showed a sharp peak, and the compounds were quantified as the integration of the peak area.

Validation of the assay

No extraneous peak was observed in the chromatograms near the peaks for the retention times of the analytes. The mass fragmentation of fatty acid methyl esters by electron ionization at 70 eV is shown in Table 1. The saturated fatty acid methyl esters and unsaturated fatty acid methyl esters showed a different tendency in the mass fragmentation. Fragments $m/z$ 74 and $m/z$ 87 were included in the mass fragmentation of the saturated fatty acid methyl esters such as methyl myristate, methyl palmitate, methyl stearate and methyl arachidate, and fragments $m/z$ 55 and $m/z$ 69 were characteristic in the mass fragmentation of unsaturated fatty acid methyl esters which contain a double bond such as methyl palmitoleate and methyl oleate. The unsaturated fatty acid methyl ester with two double bonds such as methyl linoleate and methyl oleate yielded the fragments $m/z$ 67 and $m/z$ 81. Additionally, the unsaturated fatty acid methyl ester with four double bonds such as methyl arachidonate had the fragments $m/z$ 79 and $m/z$ 91.

The limit of detection (LOD) and limit of quantitation (LOQ) of the fatty acids coupled derivatization and extraction method were calculated as 3.14 and 10 times the standard deviation obtained from the data of seven replicate measurements, respectively. The LOD and LOQ in this study were calculated in the concentration range of 0.0002–0.0011 mg/L and 0.0007–0.0035 mg/L in the control leachate, in which no analyte was detected. The high sensitivity of the target compounds by this method enables the determination of target compounds well below that reported previously.

Examination of the typical standard curve by computing a regression line of the peak area ratios of Me-fatty acids to internal standard on concentration using a least-squares fit

<table>
<thead>
<tr>
<th>Compound</th>
<th>Composition (%)</th>
<th>C14:0</th>
<th>C16:0</th>
<th>C16:1</th>
<th>C18:0</th>
<th>C18:1</th>
<th>C18:2</th>
<th>C20:0</th>
<th>C20:4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetable oils</td>
<td>9</td>
<td>0-0.1 (0.0)</td>
<td>9.7-20.3 (14.1)</td>
<td>0.0-0.0 (0.0)</td>
<td>9.9-5.8 (5.4)</td>
<td>1.9-5.8 (5.4)</td>
<td>9.7-46.8 (54.6)</td>
<td>19.7-46.8 (54.6)</td>
<td>0.5-1.9 (1.5)</td>
</tr>
<tr>
<td>Livestock manure</td>
<td>6</td>
<td>2-13.7 (4.9)</td>
<td>34.7-48.1 (42.6)</td>
<td>0-4.1 (1.1)</td>
<td>4.9-34.7 (34.6)</td>
<td>1.9-34.7 (34.6)</td>
<td>9.7-48.1 (42.6)</td>
<td>19.7-48.1 (42.6)</td>
<td>0.5-1.9 (1.5)</td>
</tr>
<tr>
<td>Carcasses leachate</td>
<td>76</td>
<td>1.0-23.3 (6.0)</td>
<td>160-702 (42.4)</td>
<td>0.1-38.0 (6.2)</td>
<td>4.9-64.6 (31.4)</td>
<td>0.2-54.1 (5.6)</td>
<td>19.7-48.1 (42.6)</td>
<td>19.7-48.1 (42.6)</td>
<td>0.1-2.4 (0.6)</td>
</tr>
</tbody>
</table>
showed a linear relationship with correlation coefficients consistently higher than 0.996 (Table 2). The line of best fit for the analytes is shown in Table 2.

Accuracy and precision were evaluated using spiked samples at concentrations of 0.2 and 1.0 mg/L. Accuracy was assessed as the relative % of the found concentration in spiked samples to the spiked concentration, and precision was calculated as their relative standard deviation. The accuracy was in a range of 87–110% and the precisions of the assay were less than 13.6% as shown in Table 2.

**Marker for carcass leachates**

Fatty acids were detected from the carcass leachates, livestock manure and vegetable oils with the established method. Eight different fatty acids were identified as follows: methyl myristate (C14:0), methyl palmitate (C16:0), methyl palmitoleate (C16:1), methyl stearate (C18:0), methyl oleate (C18:1), methyl linoleate (C18:2), methyl arachidate (C20:0) and methyl arachidonate (C20:4). From the results, vegetable oils were composed mainly of C18:2 (46.9%).

![Figure 4](https://iwaponline.com/ws/article-pdf/16/5/1287/411415/ws016051287.pdf)
C18:1 (32.9%), C16:0 (14.1%), C18:0 (5.4%), C20:0 (0.6%), C14:0 (0%), C16:1 (0%), and C20:4 (0%), and livestock manure of C16:0 (42.6%), C18:0 (34.6%), C18:1 (12.4%), C14:0 (4.9%), C18:2 (2.8%), C20:0 (1.5%), C16:1 (1.1%), and C20:4 (0%) (Table 3). Otherwise, the carcass leachates were composed mainly of C16:1 (39.8%), C18:1 (20.2%), C16:0 (15.0%), C18:2 (11.4%), C18:0 (7.5%), C14:0 (4.4%), C20:4 (1.6%), and C20:0 (0.2%) (Table 3). The main components were C18:2 > C18:1 > C16:0 > C18:0 for the vegetable oils, C16:0 > C18:0 > C18:1 > C14:0 for the livestock manure, and C16:1 > C18:1 > C16:0 > C18:2 > C18:0 for carcass leachates. From the results of the experiments, we monitored the composition of the leachates in time. The fatty acid pattern significantly varied according to the source (Table 3). The fatty acid C20:4 only occurred in the carcass leachates and C18:0 (46.9% compared with 2.8–11.4%) was much more abundant in vegetable oils than in the livestock manure or carcass leachates. Furthermore, fatty acid C16:1 was more abundant in the carcass leachates (39.8%) than in the vegetable oils (0%) and livestock manure (1.1%).

To examine the differences in fatty acids between sources, the fatty acid patterns were analyzed with multivariate statistics. In the first step, eight fatty acids, which were measured in this study, were selected as parameters, and their relative area to the highest fatty acid was obtained for each fatty acid. MDS analyses confirmed that the fatty acids from the carcass leachates were different from those from the livestock manure, and that these differences were not as pronounced as the differences between the carcass leachates and the vegetable oils. In the second step, four fatty acid groups, which were classified according to the carbon number of the fatty acids, were selected as parameters, and their relative area to the highest fatty acid was obtained for each group. Further differences between the carcass leachates of swine, bovine, and livestock manure samples appeared, and differences between the sources were most pronounced by selecting the groups (Figure 4).

MDS analysis was done on the fatty acid data to find a set of clusters. The hierarchical classification proceeded by grouping together the same carbon numbered fatty acids. The dendrogram revealed that a division in the sample groups perfectly divided them into three groups (Figure 4). The stress values were under 0.1. In the MDS analysis, the stress value gives an indication of the quality and a value of less than 0.1 is a good representation of the analyzed data (Clarke & Warwick 1994).

To find valuable biomarkers to differentiate the consequences of animal burial from vegetable oil and livestock manure, we performed eight fatty acids analyses by GC-MS. As shown in Figure 5, considerable amounts of arachidonic acid were detected only in the carcass leachates in the range of 0.005–2.2 mg/L during the first 5 months; otherwise, arachidonic acid was not found in the vegetable oil and livestock manure samples. Arachidonic acid is abundant in the brains, muscles and livers of animals (Crawford & Sinclair 1971; Nelson et al. 1997; Trappe et al. 2003); therefore, this reflects where the compound originated.
from the fast decomposition of the brain, muscles and liver of the animals in the burial pit. From the results of the fatty acid analyses, arachidonic acid is proposed as a specific indicator of carcass leachates, although it can only be detected during the first 7 months.

CONCLUSIONS

Here we presented a GC-MS determination method for fatty acids after alkali hydrolysis and acidic methylation of lipids in the leachates from animal carcasses. The method offers a very sensitive response for EI-MS (SIM). The method detection limits were 0.0002–0.0011 mg/L for fatty acids. The developed method may be valuable in the sensitive determination of specific fatty acids in groundwater and in understanding the fatty acid profile of samples.

Many livestock who have died as a result of foot and mouth disease have been disposed at landfills, and underground water contamination has been a big problem for people living near the burial sites. Until now, BOD, NH₄, total dissolved solids (TDS), chloride and potassium have been used as markers of contamination from the leachates of animal carcasses. However they also can originate from livestock manure and vegetable oils from compost. We propose in this study that the fatty acid arachidonic acid (C20:4), which is not found in livestock manure and vegetable oils from the compost of common plants, could be a specific indicator of contamination from carcass leachates. Based on the specific fatty acid composition in lipids, the fatty acid profiles of carcass leachates were also suggested to be potential indicators for contamination from carcass leachates in groundwater. MDS analyses showed that pronounced differences between carcass leachates, livestock manure and vegetable oils appeared by the grouping selected in this study. MDS analyses of fatty acids can also be used as an indicator of contamination from the leachates of animal carcasses.

REFERENCES


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