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# Chemical-Sensory Characterization of Dairy Manure Odor Using Headspace Solid-Phase Microextraction and Multidimensional Gas Chromatography Mass Spectrometry-Olfactometry

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# Chemical-Sensory Characterization of Dairy Manure Odor Using Headspace Solid-Phase Microextraction and Multidimensional Gas Chromatography Mass Spectrometry-Olfactometry

## Abstract

Livestock operations are associated with emissions of odor, gases, and particulate matter. The majority of previous livestock odor studies focused on swine operations whereas relatively few relate to dairy cattle. Identifying the compounds responsible for the primary odor impact is a demanding analytical challenge because many critical odor components are frequently present at very low concentrations within a complex matrix of numerous insignificant volatiles. The objective of this study was to describe a chemical-sensory profile of dairy manure odor using headspace solid-phase microextraction (HS-SPME) and multidimensional gas chromatography-mass spectrometry-olfactometry (MDGC-MS-O). Two analytical approaches were used: (1) HS-SPME time-series extractions (from seconds up to 20 hr) followed by gas chromatography-mass spectrometry-olfactometry (GC-MS-O) analyses, and (2) relatively short HS-SPME extractions (30 min) followed by MDGC-MS-O analyses on selected chromatogram heart-cuts. Dairy manure was collected at research dairy farms in the United States and Israel. Volatile organic compounds (VOCs) resolved from multiple analyses included sulfur-containing compounds, volatile fatty acids, ketones, esters, and phenol/indole derivatives. A total of 86 potential odorants were identified. Of them, 17 compounds were detected by the human nose only. A greater number of VOCs and odorous compounds were detected, as well as higher mass loading, on solid-phase microextraction (SPME) fibers observed for longer extractions with SPME. However, besides sulfur-containing compounds, other selected compounds showed no apparent competition and displacement on the SPME fiber. The use of MDGC-MS-O increased chromatographic resolution even at relatively short extractions and revealed 22 additional odorants in one of the regions of the chromatogram. The two analytical approaches were found to be parallel to some extent whereas MDGC-MS-O can also be considered as a complementary approach by resolving more detailed chemical-sensory odor profiles.

## Keywords

Volatile organic compounds (VOC), Odor, Solid phase microextraction 14 (SPME), Multidimensional GC-MS-olfactometry (MDGC-MS-O), Dairy manure

## Disciplines

Agriculture | Biochemical and Biomolecular Engineering | Bioresource and Agricultural Engineering | Environmental Sciences

## Comments

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1 **Chemical-sensory characterization of dairy manure odor using**  
2 **headspace solid phase microextraction and multidimensional gas**  
3 **chromatography–mass spectrometry-olfactometry**

4  
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14 **Keywords:** Volatile organic compounds (VOC); Odor; Solid phase microextraction  
15 (SPME); Multidimensional GC-MS-olfactometry (MDGC-MS-O); Dairy manure

16  
17 **ABSTRACT**

18 Livestock operations are associated with emissions of odor, gases and particulate  
19 matter. The majority of previous livestock odor studies focused on swine operations  
20 whereas relatively few relate to dairy cattle. Identifying the compounds which are  
21 responsible for the primary odor impact is among the most demanding of analytical  
22 challenges since critical odor components are frequently present at very low  
23 concentrations in a complex matrix of numerous insignificant volatiles. The objective  
24 of this study was to describe a chemical-sensory profile of dairy manure odor using  
25 headspace solid phase microextraction (HS-SPME) and multidimensional gas  
26 chromatography–mass spectrometry-olfactometry (MDGC-MS-O). Two analytical  
27 approaches were used: (1) HS-SPME time-series extractions (from seconds up to 11  
28 hrs) followed by GC-MS-O analyses, and (2) relatively short HS-SPME extractions  
29 (30 min) followed by MDGC-MS-O analyses on selected chromatogram heart-cuts.  
30 Dairy manure was collected at research dairy farms in the U.S. and Israel. Volatile  
31 organic compounds (VOC) resolved from multiple analyses included sulfur-  
32 containing compounds, volatile fatty acids, ketones, esters, and phenol and indole  
33 derivatives. A total of 86 potential odorants were identified. Of them 17 compounds

34 were detected only by the human nose. A greater number of VOC and odorous  
35 compounds were detected as well as higher mass loading on SPME fibers observed  
36 for longer extractions with SPME. Yet, besides sulfur-containing compounds, other  
37 selected compounds showed no apparent competition and displacement on the SPME  
38 fiber. The use of multidimensional GC-MS-O increased chromatographic resolution  
39 even at relatively short extractions and revealed 22 additional odorants in one of the  
40 regions of the chromatogram. The two analytical approaches were found to be parallel  
41 to some extent whereas MDGC-MS-O can also be considered as a complementary  
42 approach by resolving more detailed chemical-sensory odor profiles.

43

#### 44 **IMPLICATIONS**

45 Comprehensive chemical-sensory odor profiles should provide analysts and  
46 environmental authorities lists of target odorants to which quantification efforts  
47 should be directed. Such characterization can be useful for odor legislation as well as  
48 for the development and monitoring of odor abatement technologies. Headspace  
49 SPME combined with MDGC-MS-O is shown to be very useful to characterize  
50 odorants associated with livestock operations. Comprehensive chemical-sensory  
51 characterization can also be achieved by long SPME extractions followed by GC-MS-  
52 O analyses. Both approaches are examined in this study in order to provide detailed  
53 chemical-sensory profiles of dairy odor. To date, relatively few odor studies focused  
54 on dairy as compared to swine operations.

55

#### 56 **INTRODUCTION**

57 Confined animal feeding operations (CAFO) worldwide are associated with  
58 aerial emissions of ammonia (NH<sub>3</sub>), hydrogen sulfide (H<sub>2</sub>S), particulate matter,  
59 bioaerosols, odor and a large number of volatile organic compounds (VOC) (1-4).  
60 Some of these gases and VOC are of human and animal health concerns (5).  
61 However, many VOC produce a wide variety of unpleasant and offensive odors,  
62 which may affect the air quality and well-being of people living and working near  
63 these operations. The majority of previous livestock odor studies focused on swine  
64 operations (e.g. 6-10) whereas relatively few relate to dairy cattle (11-14). Dairy  
65 operations have multiple sources of odor emissions associated with degradation of  
66 liquid and solid manure in cattle housing, waste storage facilities, lagoons, as well as  
67 land application of wastes. A large number of VOC emitted from dairies belonging to

68 various chemical groups were identified and partly quantified in previous studies. A  
69 total of 35 compounds were identified in a commercial dairy in northern California  
70 (12), 70 compounds were identified in eight farms in northern Sweden (11), and a  
71 total of 113 VOC were resolved in a lactating open stall and a slurry wastewater  
72 lagoon in Knot Dairy in eastern Washington (14). Ammonia emissions potentially  
73 contributing to odor were monitored in Knot Dairy as well (13, 15). Previously  
74 identified VOC in dairies included acids, esters, alcohols, aldehydes, ketones, amines  
75 and other N-containing compounds, aromatic and halogenated hydrocarbons, terpenes  
76 and S-containing compounds. Many of these compounds are considered relatively  
77 mild odorants or were measured at concentrations below published odor detection  
78 thresholds (14). There is yet no comprehensive data compilation on VOC and odorous  
79 gases associated with dairy farms.

80 Chemical characterization of CAFO odors are among the most difficult  
81 analytical challenges (recently summarized by McConnell and Trabue (16)).  
82 Headspace solid phase microextraction (HS-SPME) analyses have been applied to  
83 CAFO emissions in an attempt to overcome some of the critical drawbacks associated  
84 with some other existing techniques. Sampling of whole air with evacuated canisters  
85 and air sampling bags can result in poor sample recoveries for typical malodorous  
86 gases found in livestock environments (17-19). Koziel et al. (18) showed that SPME  
87 had the highest recovery for selected 11 compounds as compared with air sampling  
88 bags, Tenax thermal-desorption tubes, and sampling canisters. Powers (20) resolved  
89 more VOC from livestock facilities by using SPME as compared with five types of  
90 solvent extraction tubes. Yet, quantification of VOC with sorbent tubes is fairly  
91 straightforward (21) whereas quantification with SPME is not simple but still possible  
92 under certain conditions (22-23). With the current state of knowledge it appears that  
93 SPME can be highly useful to resolve semi-quantitative but most detailed odor  
94 profiles.

95 Extensive characterization can be improved by simultaneous chemical-sensory  
96 analyses, because critical aroma or odor components are frequently present at very  
97 trace levels in a complex matrix of numerous insignificant volatiles. The challenge is  
98 thus to extract from a large field of 'potential' odorants, those compounds which  
99 constitute the primary odor impact that is characteristic to different livestock  
100 environments (24). Analyses utilizing GC-MS and to less extent GC-MS-O have been  
101 used in multiple studies for characterization of CAFO odor. Wright et al. (25) used

102 SPME for ambient air sampling near and downwind from beef cattle operations in  
103 Texas followed by simultaneous chemical-sensory analyses on GC-MS-O. This  
104 approach was used to isolate, identify, rank and prioritize specific malodorous gases  
105 in these environments.

106 Comprehensive odor analyses can be further advanced by the use of a  
107 multidimensional (MD) GC-MS-O. The heart-cut capability of such a system is able  
108 to resolve sections of a chromatogram, or group of peaks in a region of interest that  
109 could be difficult to separate on one column. It is achieved by connecting two  
110 columns with different stationary (e.g. non-polar and polar) phases and selectively  
111 transferring compounds from one column into the other (heart-cutting) using a flow-  
112 control switching device (25-26). Bulliner et al. (27) used the SPME and heart-cut  
113 MDGC-MS-O approach to isolate and identify specific compounds responsible for the  
114 characteristic swine odor. These findings were consistent with Koziel et al. (28) where  
115 the SPME-MDGC approach was used for ambient air sampling and analysis of air  
116 samples collected at several locations downwind from a swine operation in Iowa and a  
117 large beef cattle feedlot in Texas. Cai et al. (29) used the SPME-MDGC-MS-O  
118 method to characterize malodor partitioning to particulate matter associated with a  
119 swine barn in Iowa. Lo et al. (10) used the SPME-MDGC-MS-O approach and found  
120 nearly 300 compounds in headspace of swine manure. The SPME-MDGC-MS-O was  
121 also used to evaluate the effectiveness of zeolite in treatment odors emitted from  
122 poultry manure (30).

123 Certainly, odor information obtained by SPME (either followed by GC-MS-O  
124 or MDGC-MS-O) may be enhanced and optimized by selecting a suitable fiber  
125 coating and by using long extractions (29). Yet, long HS-SPME extractions may be  
126 applied with caution, in consideration of possible loss of information due to  
127 competition and displacement on SPME fibers exposed to concentrated complex  
128 mixtures (31).

129 The objective of this study was to describe a detailed chemical-sensory profile  
130 of dairy manure odor using headspace SPME and multidimensional gas  
131 chromatography–mass spectrometry-olfactometry (MDGC-MS-O). For this purpose  
132 we explored the information obtained by two partly parallel analytical approaches: (1)  
133 HS-SPME time-series (from 15 sec up to 20 hrs) followed by GC-MS-O analyses and  
134 (2) Relatively short extractions (30 min) followed by MDGC-MS-O analyses on  
135 selected chromatogram heart-cuts. The study was conducted on manure samples

136 which were collected from two research dairy farms in Iowa and Israel. We  
137 demonstrated how these two approaches can be used in order to enhance the level of  
138 odor characterization.

139

## 140 **EXPERIMENTAL WORK**

141

### 142 **Manure Sampling and Handling**

143 Dairy manure was collected from two research farms; the Dairy farm of Iowa  
144 State University in Ankeny, Iowa, USA ("ISU"), and the research dairy farm of the  
145 Agricultural Research Organization in Bet-Dagan, Israel ("BD"). In both sites,  
146 Holstein dairy cows were housed in free stall barns and were fed an industry standard  
147 diet. Several hundred grams of fresh manure was collected in clean glass jars,  
148 thoroughly mixed, and then multiple replicates of 3 g were transferred into clean 20  
149 ml GC glass vials (washed and baked in an oven at 110°C overnight before use). Vials  
150 containing manure samples were stored at 4°C if analyzed within a few days after  
151 collection (ISU manure) or stored at -20°C for subsequent analyses conducted within  
152 several weeks after collection (BD manure). Preliminary analyses showed that these  
153 storage conditions did not affect VOC profiles.

154

### 155 **Headspace SPME followed by GC-MS-O or MDGC-MS-O analyses**

156 Before extraction, each vial containing manure was pre-incubated for 1 h at 30°C in a  
157 water bath. The septum of the vial was then pierced using the SPME needle and the  
158 fiber was exposed to the vial headspace for the desired period of time. At the end of  
159 the extraction, the SPME fiber was retracted and the needle was removed from the  
160 vial and immediately introduced to the GC injection port. A 50/30 µm  
161 divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS; Supelco,  
162 Bellefonte, PA) was used for all analyses. Before first use, all fibers were conditioned  
163 in the heated GC injection port under helium flow according to the manufacturer's  
164 instructions. No fiber cleaning was needed between analyses if SPME fibers were  
165 reused for new extractions immediately after the previous run. Otherwise, lab-stored  
166 SPME fibers were cleaned for 16 min at an injector temperature of 230°C before use.

167 Headspace SPME time-series were performed on both ISU and BD manures,  
168 ranging from 15 sec to 11 h (ISU) or 20 h (BD) extraction time. Extractions at ISU  
169 were followed by GC-MS-O analyses whereas extractions performed on BD samples

170 were followed by GC-MS analyses only. Multiple vials were used in these SPME  
171 time-series and samples were typically extracted just once before being discarded.  
172 Separated odorous compounds eluting from the sniff port were evaluated by the same  
173 panelist in 23 out of 25 GC-MS-O runs. This panelist showed a good organoleptic  
174 capability based on an odor test presented to over 100 panelists in Israel  
175 (unpublished). A number of distinct odor events, odor character, intensity and odor  
176 duration, were recorded using a 64-descriptor panel and intensity scale in Aromatrx  
177 software (AromaTrax™ V. 6.6.1 from Microanalytics, Round Rock, TX).

178 Manure from ISU was further analyzed on a MDGC-MS-O (Microanalytics,  
179 Round Rock, TX) (10, 27, 29). The system integrates GC-O with conventional GC-  
180 MS (Agilent 6890N GC/5973 MS from Agilent, Wilmington, DE) as the base  
181 platform with the addition of an olfactory port and flame ionization detector (FID).  
182 The system was equipped with a non-polar precolumn 12 m × 0.53 mm ID (Model  
183 BP-5+, SGE, Austin, TX) and a 25 m × 0.53 mm ID polar analytical column (Model  
184 BP-20, SGE) in series with heart-cutting capability. System automation and data  
185 acquisition software were included (i.e. MultiTrax™ V. 6.00 and AromaTrax™ V.  
186 6.6.1 from Microanalytics and ChemStation™, Agilent). The general run parameters  
187 used were as follows: injector, 260°C; FID, 280°C, column oven, 40°C initial, 3 min  
188 hold, 7°C/min, 220°C final, 10 min hold; carrier gas, He. Molecular weight-to-charge  
189 ratio (m/z) range was set between 33 and 280. Spectra were collected at 6 scans/sec  
190 and electron multiplier voltage was set to 1200 V; MS ionization energy was set to 70  
191 eV. The MS detector was auto-tuned weekly.

192 The multidimensional heart-cut capability was applied on one of the  
193 interesting (i.e., associated with characteristic manure odor) regions of the  
194 chromatogram. These regions of interest were found by using the GC-FID-O mode  
195 (10, 27). Preliminary screening analyses were first completed on the precolumn, using  
196 the FID and the sniff port. Then, the most odor-complex region was selected for  
197 further characterization using 1 min width heart-cut intervals. The heart-cut selection  
198 and switching of sample flow during the run was made through the MultiTrax  
199 software.

200 The BD manure (Israel) was analyzed on a GC-MS system (GCD G1800B,  
201 HP) equipped with a 30 m × 0.25 mm ID capillary column (Rt-bDEXsm™, Restek,  
202 Bellefonte, PA). The general run parameters used were as follows: injector, 230°C;



203 MSD, 230°C, column oven, 50°C initial, 4°C/min, 150°C, 10°C/min, 200°C final, 5  
204 min hold; carrier gas, He; MS ionization energy, 70 eV; m/z range, 41-300; Scan  
205 time, 5.6/sec.

206

207

### Data Analysis

208 Compounds present in manure headspace were identified using three sets of criteria:  
209 (1) Matching of observed retention times with those of pure compounds run as  
210 standards; (2) matching mass spectrums of unknown compounds using ChemStation  
211 (ver D.00.00.38) from Agilent and NIST 98 MS spectra library (if no standard existed  
212 identifications was arbitrarily made for matching quality >70%), or BenchTop/PBM  
213 mass spectrometry library search system to resolve several chromatogram peaks in  
214 ISU samples; and (3) matching odor character (ISU only) (29). Identified compounds  
215 were semi-quantified based on peaks area counts obtained by integration of MS  
216 response with ChemStation (integration conditions: initial peak width, 0.05; initial  
217 threshold, 14). GC-MS-O and MDGC-MS-O analyses resulted in two sets of data in  
218 complementary form of total ion chromatograms (TIC) and aromagrams. The  
219 chemical signal (TIC) and sensory signal (aromagram) were then compared to find  
220 matches between odor and specific odor-causing compounds that were identified.

221

## 222 RESULTS AND DISCUSSION

### 223 Dairy manure odor profiles obtained from HS-SPME time-series followed by 224 GC-MS-O analyses

225 *Representative chromatograms-aromagrams.* Three selected simultaneous  
226 chromatograms and aromagrams from the SPME time series of ISU manure are  
227 presented in Fig. 1 (15 sec, 15 min, and 11 h extraction time). Under the same MS  
228 integration conditions, only 6 TIC peaks were recorded for 15 sec extraction, whereas  
229 114 TIC peaks were obtained for 11 h extraction time. Similarly, longer extractions  
230 resulted in a significant increase in the total number of detected odor events from  
231 separated compounds and their odor intensities. Seven characteristic odors were still  
232 detected for only 15 sec extraction, such as 'sulfuric' (methyl mercaptan), 'onion,  
233 garlic' (dimethyl sulfide and dimethyl trisulfide), 'fatty acid' (acetic acid), 'pungent'  
234 (3-methyl butanoic acid), 'phenolic' (*p*-cresol) and 'body odor' (phenolic type). Some  
235 of these chemical identifications were enabled only at longer extractions which  
236 allowed for greater mass uptake. After 11 h extraction up to 48 distinct odors were

237 perceived and recorded by the panelist. As commonly found in such simultaneous  
238 chemical-olfactory analysis (32), some apparent TIC peaks are not matched by odor  
239 peaks, and some of the odor events cannot be related to any apparent TIC peaks. This  
240 may be due to the compounds being extracted at concentrations below their odor  
241 detection thresholds but above their MS detection limit, or the compounds extracted at  
242 concentrations below their MS detection limit but above their odor detection  
243 threshold. The former is the highlighting of odorless compounds and the latter is  
244 emphasizing the usefulness of using the human nose as a detector. For example,  
245 dimethyl trisulfide (DMTS) was not detected by the MS after 15 sec of extraction but  
246 its odor was recorded by the panelist at this short extraction time ('garlic' note at  
247 RT=12.89 min; Fig. 1). Such cases eventually make the GC-MS-O approach highly  
248 valuable for comprehensive characterization of odor and specific compounds  
249 responsible for specific odors. This also illustrates cases where the use of the human  
250 nose as a detector in analytical work could be advantageous in finding and identifying  
251 of compounds that are 'overlooked' and 'missed' even with very sensitive chemical  
252 detectors.

253

254 ***Effects of extraction time on the number of compounds and mass uptake by SPME***

255 ***fiber.*** The increase in number of distinct TIC and odor peaks with increasing  
256 extraction time is demonstrated in Fig. 2a,b (ISU manure) and Fig. 2c (BD manure;  
257 TIC only). Almost a linear increase in the number of both TIC and odor peaks is  
258 observed between 15 sec and 2 h extraction time and then it continues to increase but  
259 levels off at longer extractions. The total number of TIC peaks in the ISU samples  
260 was about half of the number obtained for BD samples using the same integration  
261 conditions. Such differences can reflect differences in VOC composition of the two  
262 manures or might have resulted from the two different GC columns and MS detectors  
263 used. Nevertheless, many of these recorded TIC peaks were not identified at high  
264 quality (arbitrarily defined as >70%) and were simply counted to represent the general  
265 effect of extraction time.

266 Total mass uptake as expressed by total area counts of TIC and odor peaks in  
267 these time series are represented in Fig 2d,e (ISU) and Fig. 2f (BD; TIC only). The  
268 trends in peak area counts are similar to those obtained for the number of TIC and the  
269 separated odor events. Yet, the total TIC area counts (especially for BD samples; Fig.  
270 2f) increased quite linearly even at longer extraction time. This observation is

271 important because such linearity of uptake on SPME fibers could eventually be used  
272 for quantification of gases in the headspace, condition that the effects of sampling  
273 temperature and possibly relative humidity could be determined. On the other hand,  
274 the total odor peak area recorded in aromagrams seems to level off or even to decrease  
275 at long extractions, perhaps due to reduced olfactory sensitivity of the panelist when  
276 the fiber becomes heavily loaded with strong offensive odorants (25). Such trends in  
277 the total TIC area counts reflect both uptake of additional compounds and the higher  
278 mass loadings of the same compounds at longer extractions.

279 Several compounds belonging to major chemical classes of known offensive  
280 odorants (S-containing compounds; volatile fatty acids, VFAs; phenol and indole  
281 derivatives), were selected to show the effect of extraction time (Figs. 3a-c for ISU  
282 and Fig. 3d-f for BD manure). Phenol, *p*-cresol, indole and skatole, as well as several  
283 VFAs (mainly in ISU manure) showed a fairly linear increase in peak area with  
284 increasing extraction time. Apparently, this trend was similar in both ISU and BD  
285 manures for phenol and indole derivatives. Moreover, the slopes obtained for such  
286 compounds were quite similar for the four different compounds. The relatively small  
287 fluctuations within these trends in BD as compared with ISU samples are presumably  
288 due to the triplicate extractions made for every time point on BD samples but not for  
289 the simultaneous GC-MS-O analysis made on ISU samples.

290 The impact of extraction time on S-containing compounds was clearly  
291 different in ISU and BD manure. In the ISU samples (Fig. 3a) the peak area somewhat  
292 increased with extraction time of up to several hours, but then remained fairly  
293 constant at longer extractions. On the other hand, in the BD samples (Fig. 3d) the  
294 peak area of the same compounds continued to increase at longer extractions and the  
295 slope was substantially different for different compounds. In the BD samples, the  
296 slope (expresses the dependence of peak area on extraction time) was higher for the  
297 higher MW and the less volatile S-containing compounds (DMTS and dimethyl  
298 tetrasulfide (DMTeS)). In the ISU manure it appears that S-containing compounds  
299 reach equilibrium at long extractions. Trends differences are possibly the result of  
300 manure coming from the two locations which would cause different concentrations  
301 and composition of VOC. Thus, sorption competition and displacement from the  
302 SPME fiber (22, 29) could be affecting sampling at ISU and not in BD. [Lestremou et](#)  
303 [al. \(33\)](#) reported that some artifact sulfur compounds resulted from reactions with

304 Carboxen/PDMS fiber coating. However, the formation of artifacts cannot simply  
305 explain the differences observed between the two manures.

306 For both VFAs (ISU), phenol and indole derivatives (ISU and BD), no SPME  
307 equilibrium was observed even at the longer extractions, which is in agreement with  
308 the trend obtained for total peak area counts (Figs. 2d (ISU) and 2f (BD)). A constant  
309 increase in peak area with longer extractions excludes possible competition and  
310 compound displacement from this type of SPME fiber coating. This observation is  
311 important since it proves that long extractions can be suitably used to yield  
312 comprehensive odor characterization without displacement of at least selected  
313 offensive components.

314 Nevertheless, long extractions result in extremely complex chromatograms  
315 and aromagrams making chemical identification of multiple peaks challenging and  
316 would also make it difficult for a panelist to resolve all odor events. As shown in Fig.  
317 2, both the number of TIC and odor peaks generally increased with increasing  
318 extraction time. However, the percentage of odorous compounds (defined here as the  
319 number of odor events divided by the number of recorded TIC peaks obtained under  
320 the selected integration conditions) substantially decreased at longer extraction time,  
321 from over 100% (about the same number of odor and TIC peaks at 15 sec extraction)  
322 to about 40% at long extraction time (Fig. 4). There are two possible explanations for  
323 this trend. One is that highly volatile compounds or VOC having high affinity to the  
324 SPME fiber, are also more odorous compounds (i.e. compounds having low odor  
325 detection threshold) or as the fiber becomes heavily loaded with increasing mass and  
326 number of VOC, the panelist's sensitivity and the capability to separate odor events is  
327 diminished (human factor).

328

329 **Dairy manure odor profiles obtained from relatively short extractions followed**  
330 **by multidimensional GC-MS-O analyses**

331 Application of multidimensional GC-MS-O to characterize odorous components in  
332 livestock environments is a relatively novel approach. The heart-cut valve based on  
333 Dean's switch concept was located between the first column and the second column  
334 which was used to transfer specific pre-separated retention regions with characteristic  
335 odors from the pre-column to the analytical column. Before applying the heart-cut  
336 capability, the instrument was first set to GC-FID-O mode with no heart-cut by  
337 utilizing the sniff port to identify specific GC pre-column retention times for

338 characteristic odors of our interest (Fig. 5a). The aromagram of this GC-FID-O run  
339 resolved four odorous regions at RT=0.76 min ('sulfuric'), RT=5.2-7.6 min ('body-  
340 like', 'pungent' and 'garlic' smells), RT=10.32 min ('phenolic/medicinal'), and 16.99  
341 (not well defined by the panelist). The more complex chromatographic region  
342 (between RT of 4 and 9 min) characterized by multiple odor events was selected for  
343 further evaluation using 1 min heart-cut intervals. An example of heart-cut between 5-  
344 6 min is shown in Fig. 5b. The sample in this case was separated only on the pre-  
345 column and directly analyzed on the FID between 0-5 min. Then the selected  
346 offensive odor-causing compounds were transferred by heart-cut between 5-6 min  
347 from the pre-column to the analytical column. Only heart-cut (small segments) of  
348 chromatographic effluent that contained the characteristic odors were analyzed by the  
349 MS detector and sniff port (MDGC-MS-O mode) for further evaluation and  
350 identification. This was followed with a return to the pre-column-FID mode after 6  
351 min. All heart-cuts were completed using constant 30 min HS-SPME extractions. As  
352 shown in Fig. 5b, just one heart-cut revealed 10 odor peaks and most were not  
353 matched by TIC peaks. Such odorants, however, can substantially contribute to the  
354 total odor of dairy manure although their extracted concentrations are below their MS  
355 detection limit.

356 By applying the heart-cut capability on 1 min intervals between 4-9 min, 27  
357 odor events were resolved (events with RT between 4 and 19 are presented in Fig. 6).  
358 Of the 27 odor events only 5 distinct odors were resolved for the same extraction time  
359 of 30 min without applying the heart-cut capability (clustered odor notes in Fig. 6).  
360 Certainly some other odor events were resolved in the "GC-MS-O" mode as it  
361 included the full pre-column chromatogram and not just several heart-cuts. Most of  
362 the odor events resolved from heart-cuts were described as offensive odor, such as  
363 'body odor', 'bad socks' and 'sulfuric' ('garlic', 'onion') odor character. Notably, the  
364 heart-cut option was applied only on one of the chromatogram regions. It is expected  
365 that more coeluting compounds would be resolved if heart-cuts were systematically  
366 performed on all regions of the chromatograms (10).

367 Several of the odor events identified from heart-cuts were also resolved from  
368 applying longer extractions followed by GC-MS-O without using the  
369 multidimensional capability. This implies that long extractions followed by GC-MS-O  
370 provide some comparable information to that obtained by relatively short extractions  
371 using the multidimensional capability. Yet, the two approaches can still be considered

372 complementary for three reasons: (1) more TIC and odor events are resolved by  
373 MDGC-MS-O even for relatively short extractions as compared with longer  
374 extractions followed by GC-MS-O, (2) long extractions are not always desired  
375 because of possible biodegradation of manure samples during extraction, and (3)  
376 MDGC-MS-O could follow long extractions as well, thus potentially maximizing  
377 odor profiles resolution. Multiple 24 h extractions followed by MDGC-MS-O were  
378 applied by [Lo et al. \(10\)](#) on swine manure and 295 compounds were identified from  
379 manure headspace.

380

### 381 **Summary on Dairy Manure Odor as Resolved in this Study**

382 Table 1 summarizes the collective information resolved for ISU dairy manure odor. It  
383 consists of the results from SPME time series followed by GC-MS-O and from  
384 relatively short extractions on selected regions of the chromatogram followed by  
385 MDGC-MS-O. The table includes, in RT chronological order, all the TIC peaks that  
386 could be identified with pure standards and/or by MS spectral library. Odor events  
387 presented in the same lines match the TIC peaks. TIC identifications not matched by  
388 odor notes, and odor notes not matched by TIC peaks are written chronologically but  
389 in separate lines. VOC resolved from ISU manure included S-containing compounds,  
390 volatile fatty acids, ketones, esters, and phenol and indole derivatives. A total of 86  
391 potential odorants were identified. Of them, 33 were identified both by the GC-MS  
392 (based on their mass spectra and/or retention time of standards) and by matching their  
393 odor characters, 36 based only on their mass spectra, and 17 compounds were  
394 detected only by the human nose.

395

396 ***Comparison with previous studies.*** A large number of the VOC identified in the ISU  
397 manure was also identified in BD manure and also reported in previous studies related  
398 to dairies. Yet, substantial differences are found between the different studies. More  
399 VFAs (C2-C5) were identified in ISU than in BD manure. No VFAs were reported by  
400 [Filipy et al. \(14\)](#) and VFAs with longer C chains (C4-C12) were reported by [Sunesson](#)  
401 [et al. \(11\)](#). Same S-containing compounds were identified in ISU and BD manure.  
402 Most of these compounds were also reported by [Filipy et al. \(14\)](#) but not by [Rabaud et](#)  
403 [al. \(12\)](#) and [Sunesson et al. \(11\)](#). Similarly, multiple phenol and indole derivatives  
404 were detected both in ISU and BD manures but none of them was reported by [Rabaud](#)  
405 [et al. \(12\)](#) and [Filipy et al. \(14\)](#). More differences in other chemical groups are found

406 between these studies, including esters, alcohols, aldehydes (more compounds are  
407 reported by [Sunesson et al. \(11\)](#) and [Filipy et al. \(14\)](#)), ketons, aliphatic amines (not  
408 detected in ISU and BD but are reported by [Sunesson et al. \(11\)](#), [Rabaud et al. \(12\)](#),  
409 and [Filipy et al. \(14\)](#)), aliphatic hydrocarbons (short C chains (C3-C10) are mainly  
410 reported by [Filipy et al. \(14\)](#); longer C chains (C5-C20) mainly detected in BD  
411 manure). In general, it appears that the comprehensive approach taken in this study  
412 revealed more offensive components as compared with previous studies related to  
413 dairy odor. Besides the unique analytical approach used in this study, different animal  
414 age, nutrition diet, as well as different manure storage and management practices  
415 between dairies can cause major differences in VOC and odorants emissions.

416

## 417 **CONCLUSIONS**

418 1. Chemical-sensory characterization of dairy manure odor revealed highly complex  
419 odorants mixtures. Main organic classes included S-containing compounds, volatile  
420 fatty acids, ketones, esters, phenol and indole derivatives. One of the most causing  
421 odor regions in the chromatogram was characterized by “body odor”, “bad-socks”,  
422 “sulfuric” and “medicinal” odor.

423 2. HS-SPME time series extractions (from 15 sec up to 20 h) followed by GC-MS-O  
424 analyses revealed an increasing number of VOC and odorants. Yet, limited SPME  
425 fiber sorptive capacity and human factors can limit this approach. Also, with long  
426 extractions (hours), dynamic changes in manure and biodegradation process may  
427 interfere with characterization.

428 3. Relatively short HS-SPME extractions (30 min) followed by multidimensional GC-  
429 MS-O analyses increased resolution such that long extractions are not any longer  
430 needed for comprehensive characterization thus minimizing possible interferences at  
431 long extractions.

432 4. Overall, extraction time by SPME, multidimensional GC with heart-cut, and the  
433 desired characterization level are three elements that can be manipulated for  
434 qualitative as well as quantitative odor characterization.

435

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570

571 **List of Tables**

572

573 **Table 1.** VOC and odor components resolved in ISU dairy manure using the HS-  
574 SPME-MDGC-MS-O comprehensive analysis. The list (in RT chronological order)  
575 includes TIC-odor matches (in one line), VOC that were identified based on pure  
576 standards and/or their mass spectra (matching quality>70% except a few cases), and  
577 odor components identified by olfactometry only.

Chromatogram							Aromagram		
No	RT (min)	Compound name	CAS	Quality (%)	Std	Odor description from literature databases	Odor threshold (ppmv) <sup>++</sup>	RT (min)	Odor description by panelist
1								1.29	sewer
2	1.49	Methyl mercaptan	74-93-1	90	Y	[cabbage, sulfurous, egg] <sup>a</sup>	0.001047	1.48	feces
3	1.78	Dimethyl sulfide	75-18-3	97	Y	[cabbage, sharp, sickly, sulfurous, vegetable] <sup>a</sup>	0.002239	1.77	onion
4	1.97	Acetone	67-64-1	72	Y	[acetone, sweet fruity ketone] <sup>a</sup>	14.45		
5	3.73	2,3-butanedione (diacetyl)	431-03-8	46/63 <sup>+</sup>	Y	[butter, cream] <sup>a</sup>	0.004365	3.68	buttery
6	4.18	Isopropyl vinyl ether	926-65-8	78					
7	4.45	2-butanol	78-92-2	86	Y	[alcohol] <sup>a</sup>	1.698	4.43	sweet
8	4.74	1-propanol	71-23-8	72		[alcohol, pungent] <sup>b</sup>	2.399	4.76	grassy, sweet
9								5.21	sewer
10								5.23	body
11	5.49	2,3-pentanedione	600-14-6	47/72	Y	[almond, butter, estery apple, malt] <sup>a</sup>	0.03162	5.48	buttery, nutty
12	6.01	Dimethyl disulfide	624-92-0	97	Y	[cooked cabbage, fecal sulfurous, onion, rubber] <sup>a</sup>	0.01230	6.09	sweet
13	6.13	Ethyl butanoate	105-54-4	96	Y	[apple] <sup>b</sup>			
14								6.29	nutty
15	6.52	2-hexanone	591-78-6	60/71	Y	[fruity, ketone] <sup>a</sup> [ether] <sup>b</sup>	0.1660	6.69	solvent
16	6.82	1-butanol	71-36-3	91	Y	[floral, fragrant, fruity, sweet] <sup>a</sup>	0.4898	7.08	sweet
17								7.20	skunky
18								8.02	medicinal, garlic, onion
19	8.26	Isoamyl alcohol	123-51-3	90	Y	[whiskey, malt, burnt] <sup>b</sup>	0.04467	8.31	solvent
20								8.43	floral
21	8.53	Propyl butanoate	105-66-8	83	Y	[apple peel] <sup>b</sup>	0.03631	8.67	fragrance
22	8.70	Ethyl pentanoate	539-82-2	90		[yeast, fruit] <sup>b</sup>	0.02570	8.67	fragrance
23	9.09	2-heptanone	110-43-0	91	Y	[blue cheese, fruity, musty] <sup>a</sup> [soap] <sup>b</sup>	0.14125	9.03	spicy

24	9.20	1-pentanol	71-41-0	90		[wax] <sup>a</sup>	0.46773		
25								9.48	garlic, body
26								9.68	garlic, foul
27	9.79	3-hydroxy 2-butanone	513-86-0	86	Y	[butter, fresh, fruity, green, mould, woody, slightly rancid] <sup>a</sup>			
28	10.16	Styrene	100-42-5	91	Y	[balsamic, gasoline] <sup>b</sup>	0.1445		
29								10.23	skunky, foul
30	10.61	6-methyl 2-heptanone	928-68-7	95					
31								10.63	spicy, onion
32								10.87	medicinal
33	10.94	5-methyl 2-heptanone	18217-12-4	94					
34	11.23	3-octanone	106-68-3	94	Y	[earthy, ketone, mushroom, resinous] <sup>a</sup>	0.06026		
35								11.15	medicinal
36	11.64	2-octanone	111-13-7	91	Y	[floral, fruity, ketone, musty, soapy] <sup>a</sup>	0.01698	11.67	body, bad socks
37	12.18	1-Undecene	821-95-4	96					
38	12.21	6-methyl 5-hepten-2-one	110-93-0	96	Y	[sweet fruity] <sup>a</sup> [pepper, mushroom, rubber] <sup>b</sup>	0.03802	12.49	grassy
39	12.72	Dimethyl trisulfide	3658-80-8	97	Y	[sulfur, fish, cabbage] <sup>b</sup>	0.001660	12.69	garlic, spicy
40	12.92	3-octanol	589-98-0	90	Y	[moss, nut, mushroom] <sup>b</sup>			
41	13.08	Acetic acid	64-19-7	90	Y	[acetic, acidic, vinegar] <sup>a</sup>	0.1445	13.13	acidic
42								13.68	Bad socks, body
43	13.92	6-methyl 5-hepten-2-ol	1569-60-4	95					
44	14.01	2-nonanone	821-55-6	95	Y	[blue cheese, fatty, fruity, ketone, musty, varnish] <sup>a</sup>	0.03891		
45	14.06	3-methyl-4-nonanone	35-778-39-3	90					
46	14.48	1-Dodecene	25378-22-7	97					
47	14.53	2-ethyl-1-hexanol	104-76-7	90	Y	[rose, green] <sup>b</sup>	0.2455		
48								14.60	body
49	14.73	Propionic acid	79-09-4	91	Y	[pungent, rancid, soy] <sup>b</sup>			
50	14.90	Benzaldehyde	100-52-7	95	Y	[almond, burnt sugar] <sup>b</sup>	0.04169	14.98	Sweet, herbaceous
51	15.35	2-decanone	693-54-9	93	Y	[fruity, musty] <sup>a</sup>	0.007943		

52	15.44	2-nonanol	628-99-9	90		[coconut] <sup>a</sup> [cucumber] <sup>b</sup>			
53	15.81	Linalool	78-70-6	95		[aniseed, citrus, floral, terpene] <sup>a</sup>	0.05370	15.85	Floral
54	16.23	2-decanone	693-54-9	96		[fruity, musty] <sup>a</sup>	0.007943		
55	16.37	Butanoic acid	107-92-6	95	Y	[butter, butyric, cheese, green, musty, oily] <sup>a</sup>	0.003890	16.38	Cheeses
56	16.62	1-Tridecene	2437-56-1	95					
57								16.99	fragrance, vegetable
58	17.12	3-methyl butanoic acid	503-74-2	/63	Y	[cheese, old hop, old socks, sweaty] <sup>a</sup>	0.002455	17.14	cheese, body
59	17.34	Methyl phenyl ketone	98-86-2	90	Y	[glue, musty] <sup>a</sup> [must, flower, almond] <sup>b</sup>	0.3631	17.64	sweet, pungent
60								18.21	Medicinal
61	18.29	Pentanoic acid	109-52-4	90	Y	[dirty socks, parmesan cheese, sweaty] <sup>a</sup>	0.004786	18.36	Body
62	18.32	2-Undecanone	112-12-9	96	Y	[dusty, floral, fruity, tallow] <sup>a</sup>	0.02188	18.62	fruity, floral
63	18.75	Aniline	62-53-3	94	Y		0.6761		
64	18.88	2-butanoic acid	107-93-7	94				19.17	Offensive
65	19.46	Dimethyl tetrasulfide	5756-24-1	95		[cabbage, sulfur] <sup>b</sup>		19.55	Bad socks, body
66	20.29	2-dodecanone	6175-49-1	96		[fruity, musty] <sup>a</sup>		20.34	grassy, burnt
67	20.51	n-Pentadecanol	629-76-5	91					
68	20.65	Benzyl alcohol	100-51-6	96	Y	[sweet, flower] <sup>b</sup>			
69	21.23	2,6-dimethyl phenol	576-26-1	97	Y		0.000759		
70	21.29	Phenyl ethyl alcohol	60-12-8	93	Y	[honey, spice, rose, lilac] <sup>b</sup>	0.01698	21.49	winey, sweet
71	21.95	Dihydro beta ionone	17283-81-7	98					
72	22.06	Benzo(b)pyridine	91-22-5	93			0.01479		
73	22.31	1-Eicosene	3542-07-1	91					
74	22.59	Phenol	108-95-2	94	Y	[phenol] <sup>b</sup>	0.1096	23.13	phenolic, medicinal
75	23.19	4-methyl-2,6-di-tert-butylphenol	128-37-0	98	Y				
76	23.34	Beta ionone	79-77-6	96		[artificial raspberry, cooked carrots, floral, menthol, violet] <sup>a</sup>	0.002630		

77	23.73	<i>p</i> -cresol	106-44-5	95	Y	[medicinal, phenolic, smoky, tarry] <sup>a</sup>	0.001862	24.02	barnyard, phenolic, medicinal
78	23.83	<i>m</i> -cresol	108-39-4	96		[dry, leather, medicinal, phenolic, tarry] <sup>a</sup>	0.000794		
79	24.00	1-Octadecene	112-88-9	99					
80	25.08	4-Ethyl phenol	123-07-9	94	Y	[heavy cider, horse manure, leather, medicinal, phenolic, woody] <sup>a</sup>		25.76	barnyard, phenolic, medicinal
81	25.16	3-Ethyl phenol	620-17-7	95		[must] <sup>b</sup>			
82	25.55	Octadecane	593-45-3	95	Y	[alkane] <sup>b</sup>			
83	25.86	1-(2-aminophenyl)-Ethanone	551-93-9	97		[foxy, sweet] <sup>b</sup>			
84	26.35	3-propyl phenol	621-27-2	90				27.00	Phenolic
85	28.73	Indole	120-72-9	91	Y	[musty fecal] <sup>a</sup> [mothball, burnt] <sup>b</sup>	0.000032	29.04	barnyard, urinous, characteristic, phenolic
86	29.40	Skatole	83-34-1	91	Y	[mothball, fecal] <sup>b</sup>	0.000562		

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(+) Identification quality obtained by ChemStation (left) and by BenchTop/PBM mass spectrometry library search system (right).

(++) From Devos et al. (34).

(a) LRI database (<http://www.odour.org.uk/odour/index.html>, accessed on June 17,2006)

(b) Flavornet database (<http://www.flavornet.org/flavornet.html>, accessed on June 17, 2006)

Std = confirmed with pure standard.



585 **Figures captions**

586

587 **Fig. 1.** Typical TIC and aromagrams of ISU dairy manure obtained by SPME time  
588 series followed by simultaneous chemical-olfactory analysis. The units on the Y-axis  
589 are relative intensities based on the MS response (TIC) and the odor intensity  
590 recorded using AromaTrax software (the baseline of the aromagram is arbitrarily  
591 determined).

592

593 **Fig. 2.** The effect of SPME extraction time on the number and total area counts of  
594 TIC and odor peaks resolved by SPME time series followed by simultaneous  
595 chemical-olfactory analysis (ISU manure; a, b, d, e) or by chemical analysis only  
596 (BD; c, f). Error bars (BD samples) represent the standard deviations of triplicate  
597 analysis.

598

599 **Fig. 3.** The effect of SPME extraction time on the total TIC area counts obtained for  
600 selected compounds representing three major groups of known offensive odorants  
601 associated with livestock production. Results are presented for ISU dairy manure (a-c)  
602 and BD manure (d-f). Error bars (BD samples) represent the standard deviations of  
603 triplicate analysis.

604

605 **Fig. 4.** The number of odor peaks relative to the number of TIC peaks (% of  
606 "odorous" peaks) resolved from SPME time series followed by simultaneous  
607 chemical-olfactory analysis.

608

609 **Fig. 5.** A simultaneous chemical-olfactory screening using the non-polar pre-column  
610 and the FID detector (a). This analysis was used to select regions of interest for  
611 further comprehensive analysis using the multidimensional heart-cut capability. A  
612 MDGC-MS-O output includes the FID response with the heart-cut region (5-6 min)  
613 shown as a straight line, and the simultaneous TIC-odor analysis on the selected heart-  
614 cut that is further separated on the polar column.

615

616 **Fig. 6.** The collective odor information resolved by a constant SPME extraction time  
617 of 30 min, using a full run (no heart-cuts) and using five heart-cuts of 1 min intervals,  
618 made on FID retention time between 5-9 min. This region was selected to elucidate

- 619 odor components in one of the most complex odor region of these chromatograms.
- 620 Odor notes were made by the panelist using AromaTrax.