

Characterization of Volatile Organic Compounds and Odors by *in vivo* Sampling of Beef Cattle Rumen Gas Using Solid Phase Microextraction and Gas Chromatography-Mass Spectrometry-Olfactometry

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Summary and Implications

Volatile organic compounds (VOCs) and odors in cattle rumen gas were characterized using *in vivo* headspace sampling with solid phase microextraction (SPME) coupled with gas chromatography-mass spectrometer-olfactometry (GC-MS-O) analysis. A novel device allowing for headspace SPME (HS-SPME) sampling through the cannula was designed, refined, and used to collect rumen gas samples from steers. Carboxen/polydimethylsiloxane (PDMS) fiber (85 μm) was used in the SPME sampling. Fifty VOCs belonging to 10 chemical functional groups were identified in the rumen headspace. The identified VOCs had a wide range of molecular weight (MW) (34 to 184), boiling point (-63.3 to 292 $^{\circ}\text{C}$), vapor pressure (1.05×10^{-5} to 1.17×10^2 Pa), and water solubility (0.66 to 1×10^6 mg/L). Twenty-two compounds have a published odor detection threshold (ODT) of less than 1 ppm. More than half of the identified compounds are reactive and have an estimated atmospheric lifetime of < 24 hr. The amounts of VFAs, sulfide compounds, phenolics, and skatole, and odor intensity of VFAs and sulfide compounds in the rumen gas were all higher after feeding than before feeding. These results indicate that rumen gases can be an important potential source of aerial emissions of reactive VOCs and odor. *In vivo* sampling via SPME coupled with GC-MS-O analysis can be a useful tool for qualitative characterization of rumen gases, digestion, and its relation to odor and VOC formation.

Introduction

Rumen headspace is saturated with compounds produced during digestion. Composition of ruminal fluid has implications on the digestion processes. Thus, chemical composition of ruminal fluid is important for nutritional studies. Feed utilization and feed additives can have an impact on odor and gas emissions from manure. Rumen gas can be released to atmosphere via eructations and exhaled breath. Digested products from rumen can also be released

with manure and therefore be a source of aerial emissions of VOCs and odor.

Characterization of fermentation products is used in assessing the extent and nature of microbial fermentations. To date, nearly all studies focused on the characterization of ruminal fluid itself. Relatively little is known about the composition of rumen gas and its implications for gaseous emissions. Sampling of gas instead of liquid is more challenging from an analytical standpoint. However, one benefit is a minimization of multiphase liquid-solid sample matrix which requires extensive sample preparation. Measurement of gases produced by rumen microbes could be very useful in evaluating diets, animal health status, feed additives, dietary amendments, and rumen fermentation.

To date, no olfactometry analyses were reported on rumen liquid or gas in previous studies. Odor analysis could provide additional insight to the specific makeup of gas, particularly in some cases where the human nose is more sensitive than conventional analytical detectors. Thus, the link between specific diet, rumen gases, and livestock odor warrants research.

This study was conducted to characterize volatile organic compounds (VOCs) and odors in cattle rumen gas through *in vivo* sampling of the rumen gas. In this research, a novel device allowing for headspace SPME (HS-SPME) sampling through the cannula was designed, refined, and used to collect rumen gas samples from steers. Rumen gas samples were analyzed using a GC-MS-Olfactometry system allowing for simultaneous VOCs/odor qualitative characterization. To our knowledge, this is the first investigation of this kind to conduct *in vivo* SPME and to evaluate rumen gas odor.

Materials and Methods

A novel device (Figure 1) allowing for headspace (HS) SPME sampling through the cannula was designed, refined, and used to collect rumen gas samples from three steers for three days. This device uses a cannula stopper modified with a sealed septum port for insertion of SPME fibers.

Carboxen/PDMS SPME fiber (85 μm) (Supelco, Bellefonte, PA, USA) was used for rumen headspace gas sampling. Three rumen cannulated (101 mm I.D.) Angus steers (868 ± 49 kg body weight) were individually restrained in a hydraulic chute during the SPME sampling. Multidimensional GC-MS-O (Microanalytics, Round Rock, TX, USA) was used for all analyses. Human panelists were

used to sniff separated compounds simultaneously with chemical analyses.

Results and Discussion

The new device proved useful for *in vivo* rumen gas collection with SPME in field conditions. Sampling times as long as 10 min were practical. Longer extraction times may be possible with free-ranging steers if the septum port is protected.

SPME-GC-MS-O can be a useful technique to monitor feed digestion *in vivo* and to observe the relationship between feed and odor/VOC emissions from beef cattle operations.

Rumen gas contains at least 50 VOCs belonging to 10 chemical functional groups. In this research, 34 were confirmed with pure standards. Identified compounds had a wide range of MW, boiling point, vapor pressure, and water solubility. A new chemical group found in rumen gas was monoterpenes.

Many of the most offensive and characteristic odorants associated with livestock production were found in rumen gas. Odorous gases included those emitted from manure

such as VFAs, VSCs, phenolics, and indolics. As many as 22 compounds had an ODT < 1 ppm. These results indicate that rumen gases could be a source of aerial emissions and odor. Amendments to the rumen environment could potentially have implications to odor control.

More than half the rumen gas compounds identified in this research are reactive and have an estimated atmospheric lifetime of < 24 hours. At least one gas (dimethyl sulfide) is suggested as an inverse 'greenhouse effect' gas. More research is warranted to determine actual concentrations and emissions of these rumen gases to the atmosphere as they may be important odor sources in areas with large cattle populations.

Acknowledgements

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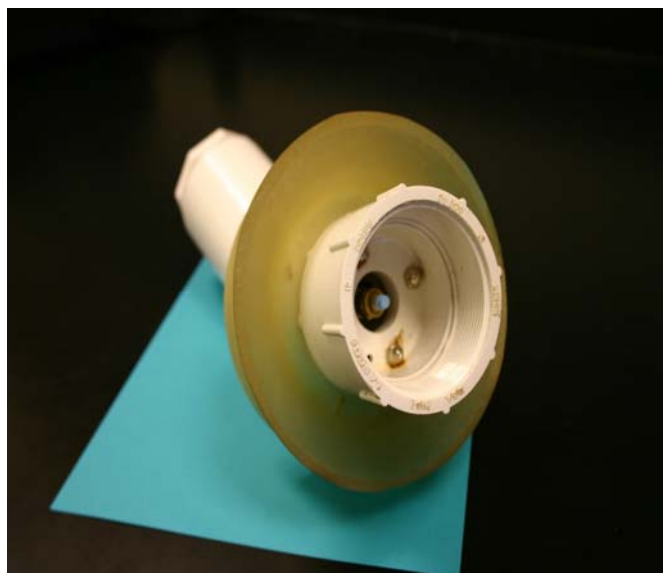


Figure 1. Photo of the refined device with SPME allowing for headspace (HS) SPME sampling through the cannula.



Figure 2. Photo of sampling the gas in the headspace of rumen with SPME.

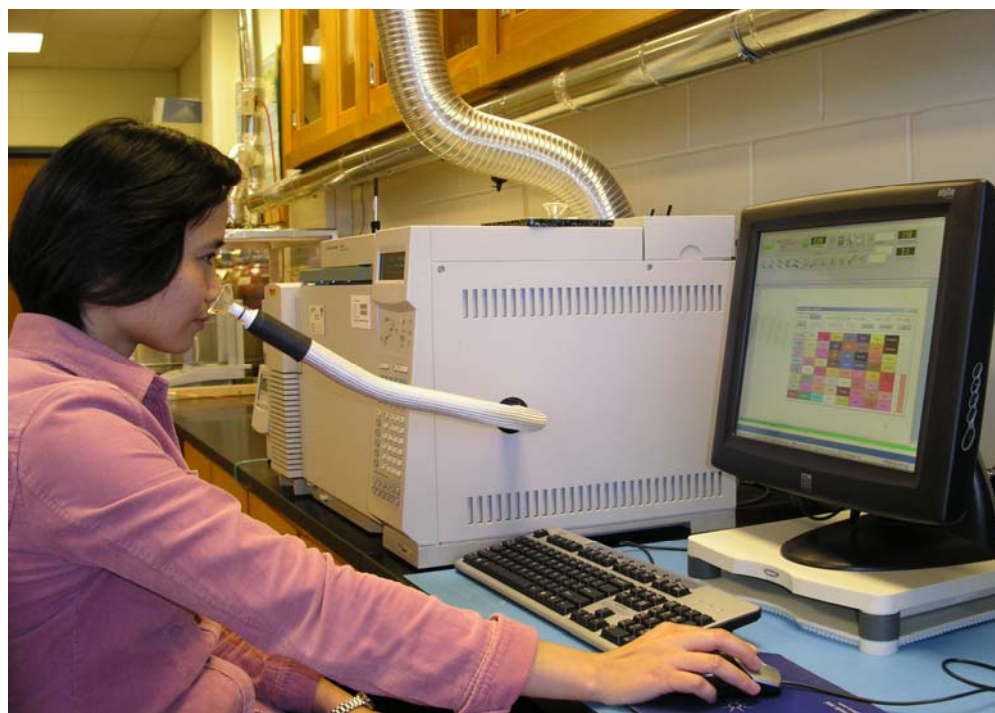


Figure 3. Panelist evaluating odor of rumen gas.

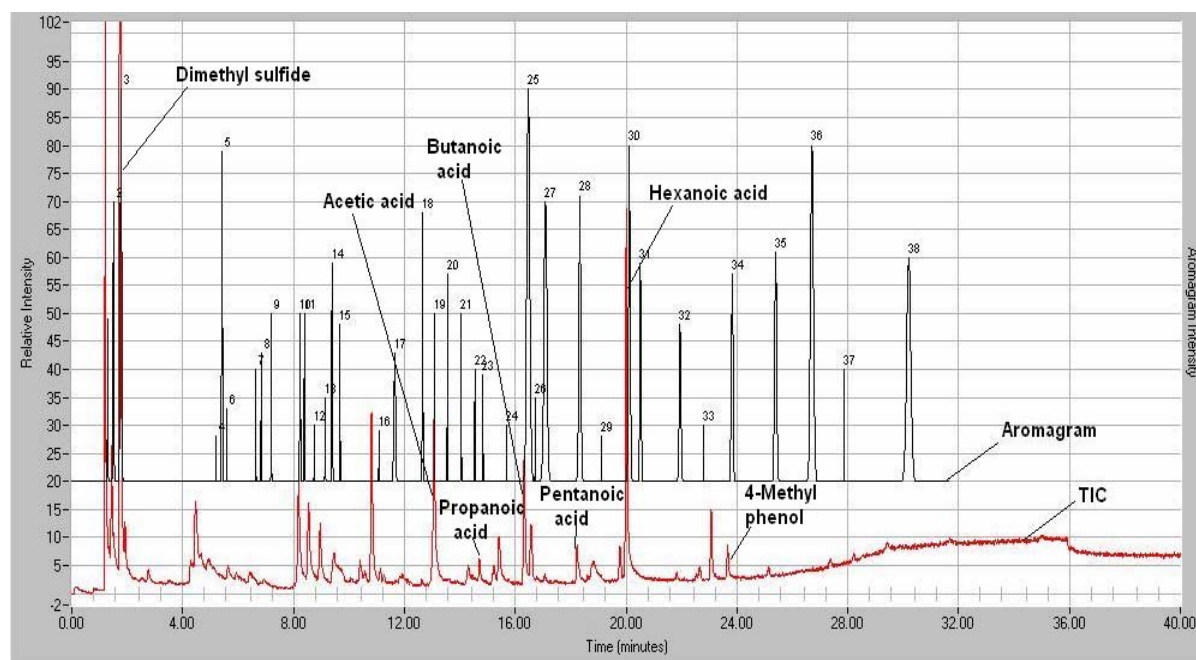


Figure 4. Total ion chromatogram (TIC) (lower, red line) and aromagram (upper, black line) of rumen gas after feeding. Samples were collected using Carboxen/PDMS 85 μ m SPME fiber and 5 min *in vivo* rumen sampling time.