RESEARCH REPORT

NPB FINAL RESEARCH GRANT REPORT

Project Title and NPB project identification number: "Can carcasses of pigs euthanized with foam be rendered" NPB #22-085
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Industry Summary: The completed study provided information on the ability of foamed pig carcasses to be rendered. We obtained three foams, Foams A, B, and C, used for the humane killing of groups of animals in emergencies, i.e., foams used for depopulation activities. We evaluated the foams for components that could be potential residues in rendered products using mass spectrophotometry and light chromatography (MS/LC) since MS/LC is an accepted method used in food production/food safety laboratories to detect and characterize contaminants and residues, such as antibiotics and chemical growth-promoting agents. After optimizing the LC/MS method for the foam components, we humanely killed three groups of pigs with nitrogen-gas-filled foam, one group for each Foam A, B, and C, n=3 pigs per group. An additional pig (n=1) was humanely killed by captive bolt and served as a negative (no foam, i.e., no residues) control. After confirmed death and transportation to the rendering laboratory, we collected, measured, and weighed sections of skin, snouts, ears, and legs (external tissues/areas of the pig carcass in contact with the foam) from all carcasses. We determined if cooking at temperatures, times, and conditions that mirror the rendering process eliminated the residues from the collected tissues in contact with the foam. There were no detectable foam residues after rendering tissues from carcasses exposed to two of the foams (Foam A, plant-based; Foam C, Green-certified, Class A/B firefighting foam) and only minimal levels detectable for Foam C (a class A firefighting foam). Having rendering as a disposal option will not only help in the adoption of using foam-based humane killing and depopulation methods in the U.S. but also provide a cost-recovery option for producers that have depopulated pigs via foam methods by allowing the carcasses to be rendered rather than composted.

Key Findings:

- Please provide 3 to 5 bulleted points that highlight the most important findings of your study
- These can most simply be distilled from the "Discussion" section
- They should be written to convey information to pork producers
- Be brief

Keywords: foam, carcasses, depopulation, rendered, disposal

Scientific Abstract: This research project addressed a critical knowledge gap surrounding the disposal of pig carcasses subjected to gas-filled or water-based foam depopulation methods in the United States. Since the rendering industry's hesitancy to accept foam-depopulated pig carcasses is due to uncertainties about potential residues in rendered products, three foams were fully characterized to find compounds that were suitable as markers for the detection of foam residues in pig tissues. The foams were then used to humanely kill experimental groups of pigs with nitrogen-gas-filled foam. Tissues from foamed pig carcasses, snout, ears, skin, and feet, were collected, weighed, and cooked at times, temperatures, and conditions to simulate rendering to determine if any residues persist in rendered products. To determine if cooking eliminated or reduced the levels of foam, as ascertained by the detection of the suitable identified ion chemical markers, samples were tested in triplicate as raw and after cooked. Cooking was conducted in a commercial stove in a meat science laboratory at conditions that mirrored rendering, with tissues being primed with hot yellow pork grease and cooked to pathogen-killing temperatures for a sufficient duration (130°C (265°F) for 75 minutes). Raw samples and cooked tissues, after removing from fat, were extracted, and tested for the presence of the chemical ion markers using light chromatography-mass spectroscopy (LC-MS) at a food research laboratory. Levels obtained by LC-MS were adjusted for tissue weights to calculate mg/g of the identified marker of foam/tissue. Results of LC-MS demonstrated that after cooking tissues at conditions equivalent to rendering, foam residue chemical marker levels were reduced 3-fold, often to levels below detectable

limits. Positive control ear sections soaked in water-based foams had detectable foam residue chemical markers at minimal levels in the raw samples that were reduced or non-detectable after cooking. There were low or non-detectable levels of foam residue chemical markers in both the raw and cooked samples for two of the three foams. After cooking, the skin, the largest organ of the body and the organ in most contact with foam, from foamed carcasses, had levels that ranged from non-detectable (*) to 0.02 (0.01 mg/g average). Since the majority of the cooked samples tested had no to minimally detectable chemical markers of foam residues, it may be possible in the future to implement procedures to prevent even low-level foam residues, such as rinsing the external surfaces of the carcasses of pigs humanely killed by foaming before grinding and rendering. If carcasses of foamed pigs can be rendered, the cost-savings and resource-sparing would be substantial and limit the additional losses due to depopulation by providing an alternative method of disposal.

Introduction: The objectives of the research project were to fill a knowledge gap regarding the disposal of carcasses of pigs depopulated via gas-filled or water-based foam depopulation methods. The renderers in the United States have not yet accepted carcasses from pigs depopulated with foam since it is currently unknown if there are components in the foam that may result in residues in rendered products. Therefore, we proposed to fully characterize foaming compounds used for mass depopulation via gas-filled or water-based foam and determine if any residues from the compound of the foam would persist in rendered products.

If the rendering process eliminates all traces of the foam compounds, carcasses of pigs depopulated with foam, i.e., foamed carcasses, could be rendered which would allow some cost-recovery both to those companies/producers who had to sacrifice these animals and to the regulatory agencies indemnifying the depopulation. Furthermore, if foamed carcasses could be rendered rather than composted or incinerated, the fallow period of the farm could be shortened, which also decreases the cost of lost production.

Objectives:

1. To identify chemical markers of foam products used for mass depopulation via gas-filled or water-based foam.
2. To determine if any foam residues would still be present in raw and rendered pork tissues through chemical markers.

Materials & Methods:

<u>Foam</u>

Three foams were used. Foam A was a plant-based oil soap product imported to the USA for use in humane-killing by nitrogen-gas-filled foam anoxia research at the University of Minnesota (USDA APHIS NADPRP project AP21VSSP0000C030, "Adapting High Expansion Foam for Use in American Systems for Mass Depopulation and On-Farm Culling with UMN IACUC Protocol 2101-38763A). Foam B was a class-A firefighting foam concentrate. Foam C was a GreenScreen-certified, class A/B fire-fighting foam concentrate. All foams were chosen because they were PFOS/PFAS not-detected (results not shown) and Foams B and C are also used for HPAI-related poultry depopulation activities or depopulation research in the Midwest. To quantify the volume of foam deposited on an ear section and to provide samples of foam for liquid chromatography-mass spectrometry (LC-MS), the three foam concentrates were diluted in 50 ml sterile polystyrene centrifuge tubes (tubes; Corning Centri Star 430829) as follows: Foam A = 2 ml foam in 40 ml tap water to make ~5% solution; Foam B = 0.4 ml foam in 40 ml water to make ~1% solution; Foam C = 0.4 ml foam in 40 ml water to make ~1% solution. Tap water (O) used to make the foam solution was also saved and submitted for LC-MS. From each foam solution, 10 ml was poured into three tubes (A1, A2, A3, B1, B2, etc) to soak the pig ear samples in foam. Soaking was done to simulate carcass conditions after humane killing by nitrogen-gas-filled foam.

<u>Pig ear samples for soaking in water-based foam, quantifying the amount of foam (or water) deposited on the ear, and creating positive controls for raw and cooked samples</u>

Pig ears were obtained from dead pigs at privately owned farms, from slaughter plants, or retailers. Each pig ear was rinsed with tap water and dried with paper towels before sample extraction. Samples extracted from the ears were 2 cm X 2 cm then further trimmed to a final weight of 1.0 (one) gram per weighing with a CEN-TECH 93543 Digital Scale with a precision of 0.1 gram. A one-gram section of the ear was placed into each tube. There were three tubes per foam and three tubes for water to provide 12 "soaked" ear samples (three per foam A, B, and C as positive controls and water O as negative controls). The tubes containing 10 ml water-based foam solution (A, B, C) or tap water (O) and 1 section of the ear were shaken for 1 minute using VWR Vortexer 2 at maximum setting and then allowed to sit for 7 minutes to soak (to simulate dwell time in foam-filled containers used for humanely killing pigs). After the soak time was completed, dry forceps were used to extract the ears section and placed directly into the labeled weigh boat.

Weigh boats (VWR Cat. No 10770-450 140X140X22 mm PS Large) were used and the labeled weigh boat was used for ears pre- and post-soak for A, B, and C. Made 3 foam dilutions in 50 ml sterile polystyrene centrifuge tubes (tubes; Corning Centri Star

430829). All ear sections had foam residue on them when placed in the weigh boat for post-foaming weight and the WATER (O) ears were wet.

The ear-soaking process was conducted on two additional pieces of 2 cm X 2 cm sections of the ear. The sections were placed into prepared centrifuge tubes containing 10 mls of liquid (foam solutions A, B, C, or Water). The tubes were closed and shaken manually for 30 seconds. After 30 seconds, the ears were allowed to soak for 7 minutes to simulate the dwell time. After 7 minutes, one of the pieces of the soaked ears was placed in a separate, clean 50 ml centrifuge tube for "raw" LC/MS residue readings and the other was placed in a round-bottomed glass tube for cooking. A total of three replicates of soaking ears were completed resulting in 36 ear skin samples (18 raw, 18 cooked).

Humane-killing of pigs by nitrogen-gas-filled foam anoxia (Groups A, B, and C) or by captive bolt (Group O)

Three groups (Groups A, B, and C humanely killed by gas-filled foam using Foams A, B, and C respectively) of three pigs each were humanely killed via N-2 gas-filled foam anoxia following protocols described in UMN IACUC Protocol 2101-38763A. A control pig, Group O, n=1, was humanely killed with a non-penetrating captive bolt by the farm manager following the farm's euthanasia action plan written by the farm's attending veterinarian. All pigs were cull pigs obtained from a privately-owned farm, were on average 24 days of age, and weighed on average 5 kg (range 3 to 7 kg). Pigs in Groups A, B, and C were humanely killed in a container (600 x 400 x 375 mm) made with high-density polyethylene (HDPE) agricultural plastic walls, sides, and top designed and made by the company HEFT AB (heftinternational.com). The internal capacity of the container was .33 square meters or approximately 3.5 square feet. The lid on the top of the container was opened and three pigs per group were manually loaded into the container from the top. A solution of water and 5% foam agent A was used for group A. For pigs in Groups B and C, a one-percent solution of Foam B and Foam C were used, respectively. The foam produced contained >99% purity of nitrogen gas. The pigs were observed throughout the process through the transparent, plexiglass lid. For all groups, the container was filled with nitrogen-gas-filled in less than 30 seconds, anoxic conditions (<2% oxygen) were achieved and held for 420 seconds (seven minutes) as measured by two oxygen sensors (NeuLog Oxygen logger sensor NUL-205; neulog.com). During the 420 seconds dwell time, pigs lost posture in less than 50 seconds and all movements ceased at 140 seconds on average. All pigs were confirmed dead after 480 seconds by the veterinarian's post-mortem examination which confirmed no audible heartbeat, no breathing, no corneal reflex, and no reflex to pinch stimulus of digits. Pig carcasses were placed in sealed plastic bags by group. Bags of pig carcasses were transported on ice gel packs in coolers for 24 hours while in transit to the rendering laboratory for sectioning and rendering.

Sample Collection from Pig Carcasses

At the rendering laboratory, from all twelve pigs, the samples listed in Table 1 were obtained using individually cleaned, disinfected, and dried tools.

Table 1. Samples collected from carcasses with descriptions of anatomic location, amount, and tool used

Sample	Amount/Description	Tool used
Snout	rostral 0.5 to 1 cm	bone cutters
Legs	distal 6 cm of the front and rear legs	bone cutters
Ears	4 X 4 cm	scissors
Skin	10 X 10 cm from neck to flank	scissors

Snout, Legs, ears, and skin were then sectioned by scissors and knives into 3 cm sections (maximum size) and placed into individually labeled clean plastic storage bags. Storage bags were labeled by foam and pig # (Pig 1-Foam A-Ear; Pig 2-Foam A-Ear, Pig 3-FoamA-Ear, Pig1-Foam A-Legs, etc.). Samples were divided equally into beakers for cooking at rendering equivalent conditions to serve as rendered samples or into labeled tubes to be refrigerated and frozen and serve as raw samples (hereafter referred to as raw and cooked samples). The time lapse between dissection and cooking was 20 hours to 44 hours. All samples were held at 4C until cooking and/or further sampling by LC-MS. Additionally, saved from each pig were pluck, i.e., tongue, trachea, esophagus, cardiopulmonary vessels, lung, and heart) and stomach with contents.

Raw sample refrigeration and freezing conditions

A minimum of 10 grams of each sample was saved in 50 ml sterile, polypropylene disposable centrifuge tubes, Fisherbrand® cat no 05-539-8, and held at -20C frozen until analysis by LC-MS.

Cooked samples rendering equivalent conditions

Snout, leg, ear, and skin samples for cooking were removed from storage bags, weighed with an ACCUTECK Digital scale, model WA260A6GOLD, and placed into individual 100 ml KIMAX ® glass beakers. To each beaker, yellow pork grease, supplied by Tyson Foods, was added for priming. A single 100 ml beaker was filled with 40 ml of yellow pork grease only and a temperature probe, HUATO ® model: S220-T8 (CE), was inserted in the yellow grease only beaker to ensure that the grease melted and cooked within a temperature range of 50 to 82°C (120 to 180°F).

To simulate rendering, cooking was performed at the following conditions which were determined after consultation with rendering experts in North America. The maximum sample size was a 2 cm X 2 cm section to simulate the maximum rendering grind size of carcass material of 3.5 cm. The temperature of the Vulcan Commercial Cooking Oven, model no VC4ED, DEV.NO 11D3, SERIAL NO 481900771, was set to 140°C (285°F) with a fan speed of low. The samples were placed into glass beakers containing 4 parts of sample to 1 part of yellow grease. The beakers containing samples + yellow grease were placed on an aluminum baking tray (45 cm X 60 cm "large" tray) lined with aluminum foil into the preheated oven. After the samples were placed in the oven, the oven temperature was raised to a setting of 177°C (350°F) to quickly heat the beaker of yellow pork grease only to 130°C (265°F) within 15 minutes. To monitor temperatures, one temperature probe was placed in the oven and the other probe was placed in the glass beaker containing only yellow grease. When the pork grease reached 130°C (265°F), a 75-minute timer was set and the temperature was read every 10 minutes to make sure that the samples and grease remained at 130°C (265°F) with the oven temp reading approximately 5°C (10°F) higher, i.e., the oven temperature was consistently 135°C (275°F) when the yellow grease temperature was 130°C (265°F). The oven temperature was regulated down to 149°C (300°F) but always within the range of 130°C (265°F) to 149°C (300°F). After 35 to 45 minutes of cooking, all beakers were stirred manually with a knife. Knives were switched groups (Foam A, B, C, Water) but not between sample types (Figure 1). After all samples were stirred, the samples were placed back in the oven and the timer restarted. After the samples were cooked at 130°C (265°F) for 75 minutes total, samples were removed from the oven, and the fat was immediately decanted as hot fat into a 50 ml polypropylene centrifuge tube, individually labeled by pig, foam type, and sample type. The crax, i.e., the cooked/rendered tissue, was transferred hot into a separate individually labeled tube. All fat and crax tubes were cooled at room temperature and held at room temperature for a maximum of 24 hours. All samples after cooling were placed into a freezer and held at 20C until samples could be extracted and tested by LC-MS. All samples were tested in triplicate.

Figure 1. Stirring of cooked samples halfway through the cooking process.

Sample extraction for liquid chromatography-mass spectrometry (LC-MS)

Raw and cooked samples were extracted using a water-soluble compound extraction method published by To et al. (2023). The selection of water-soluble fraction to be analyzed for foam markers was evaluated through a series of extractions of foam solutions and pig ears dipped in foam solutions, compared negative controls (distilled water), using various solvent combinations. These extracts were analyzed by both LC-MS and GC-MS scanning methods and evaluated for both peak and ion intensity and consistency. Water-soluble fraction delivered the most consistent intensity of markers across extractions by LC-MS method. This finding was also confirmed by the water-soluble nature of all three foams.

Tissue weight varied in weight depending on anatomical location and treatment (raw or cooked) because cooked samples tended to have much less residue, thus the sample weight was much greater. After being weighed into 50-mL polypropylene centrifuge tubes, samples were mixed with 0.4 M perchloric acid to inactivate enzymes and precipitate proteins. The water-soluble compounds were extracted in LC-MS water and acetonitrile. The extracts were then neutralized by potassium bicarbonate, filtered through 0.2-µm nylon membranes, and injected into an Agilent LC-MS Triple-Quad system.

Liquid chromatography-mass spectrometry (LC-MS)

Foam samples were submitted to Tyson Foods WBA Analytical Laboratories for identification of the most abundant ions present in the foams by liquid chromatography–mass spectrometry (LC-MS). Table 2 lists the three ions identified by LC-MS that were used as quantitative markers for the detection of foam residues on the tissues.

Table 2: Ions used as quantitative markers for Foam A, B, and C

Foam products	Ion 1	Ion 2
Foam A	m/z 353.2/RT 3.8 min	m/z 354.2/ RT 3.8 min
Foam B	m/z 214.8/RT 0.58 min	m/z 138.9/RT 1.26 min
Foam C	m/z 214.9/ RT 1.1 min	m/z 215.1/RT 1.1 min

Additionally, ions were identified that differentiated foam-positive samples and foam standard solutions from negative control, i.e., water or tissues from carcasses of pigs humanely killed by captive bolt. After shot-gun profiling, the sample quantity was increased, and chemical markers were monitored for correlation with sample weight. Best correlated chemical markers were selected and spiked in raw and cooked pig tissues for determining matrix effects. The LC-MS method was validated for levels of detection and quantification, linearity, precision (repeatability and reproducibility), and accuracy (% recovery).

The LC-MS method was developed to identify the most abundant ions that differentiate foam-positive samples and foam standard solutions from negative control (tissues free of foams). The pig ears were collected to create 1-g pieces that were randomly divided into negative control and positive control (dipped in 5, 1, and 1 % solutions of foam A, B, and C, respectively). Three sets of samples (negative control, positive control, and foam standard solutions) were extracted and analyzed in a scan mode to determine unique peaks and ions that only exist in positive control and foam standards.

Figure 2: Examples of ions that differentiated the same peaks of Foam A residue.

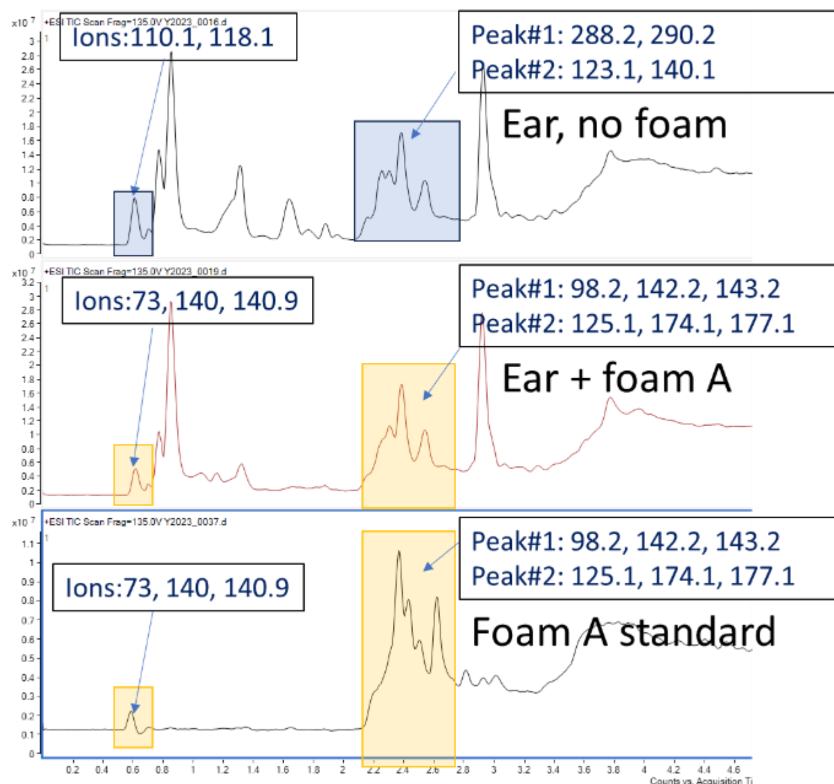


Figure 3: Examples of ions that differentiated the same peaks of Foam B residue

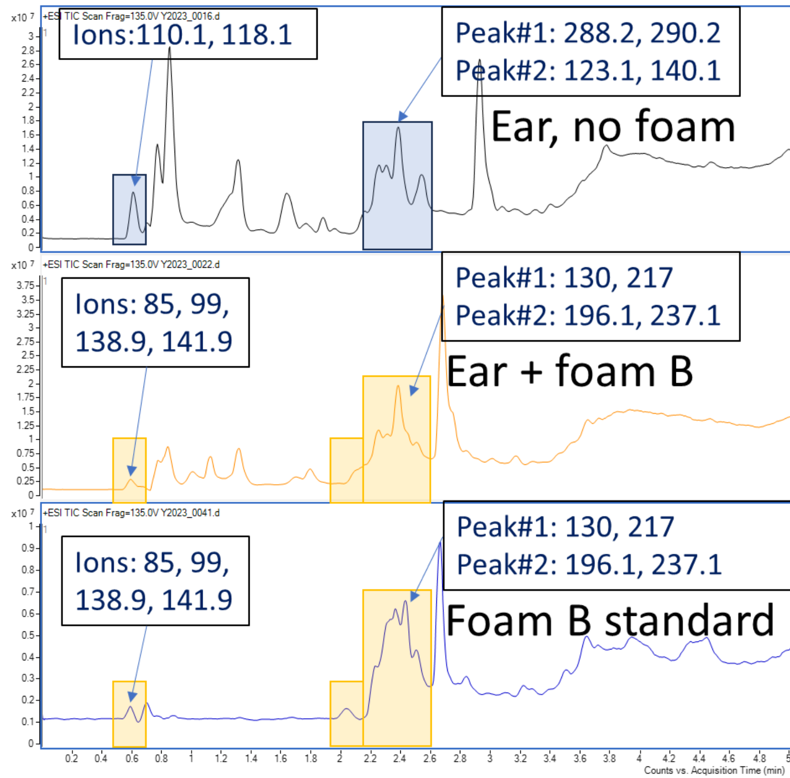
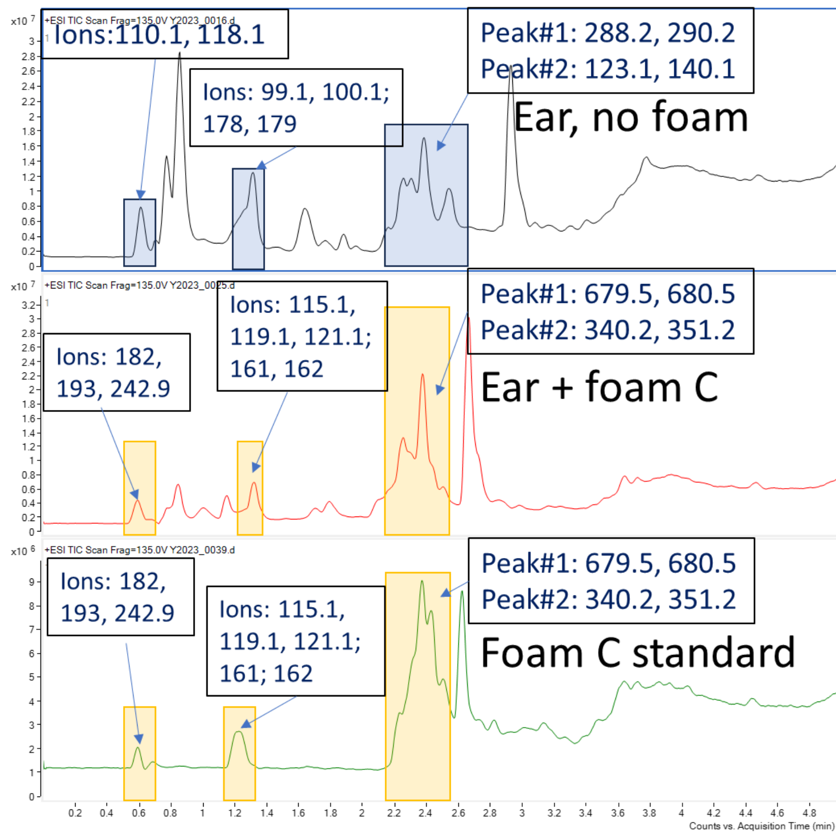


Figure 4: Examples of ions that differentiated the same peaks of Foam C residue



This process, including testing various tissues and concentrations, extracting predominant ions at various retention times, and validating these ions for applicability (being absent in blank samples, repeatable and reproducible, and linearly increasing with foam concentration) resulted in specific ions being quantified in a selective ion monitoring (SIM) mode. The mass spectrometry collision energy was optimized for the selected target ions in MassHunter Acquisition Software (Agilent Technologies Inc, Santa Clara, USA). The two most abundant ions, as shown previously in Table 2, were used for the quantification of foam content on tissue surfaces.

The LC-MS system includes an Agilent Triple-Quad 6470B Mass Spectrometer coupled with an Agilent 1290 Infinity II series UPLC system (Agilent Technologies Inc, Santa Clara, USA), supplemented by a PEAK Genius XE35 nitrogen generator (Scotland, UK). Samples were injected at 10-µL and at a constant column temperature of 40°C. Compounds were separated through a Zorbax C18 reversed-phase column (150 mm × 4.6 mm, 3.5-µm particle size) by a gradient program of two mobile phases, water with 0.1% (v/v) formic acid (eluent A) and acetonitrile (eluent B) at a flow rate of 0.5 mL/min. The gradient phase composition is shown in Table 3.

Table 3: Solvent gradient to elute marker compounds for foam A, B, and C

Time (min)	Eluent A (%)	Eluent B (%)
0.00	100	0
0.50	100	0
1.50	60	40
3.00	60	40
3.50	0	100
4.50	0	100
4.60	100	0
6.00	100	0

The mass spectrometer was operated at a drying gas temperature of 300°C, a drying gas flow rate of 10 L/min, a nebulizer pressure of 25 psi, a nebulizing gas temperature of 350°C, a nebulizing gas flow of 11 L/min, and a capillary voltage of 4000 V.

Calibration and calculation

The standard foam solutions were prepared freshly by diluting the concentrated foams with tap water, similar to how they were prepared for euthanasia. The concentrations range from 0 to 5% for foam A and 0 to 1% for foams B and C. One gram of squared pig ear piece was placed into each solution, vortexed for 30 s, and soaked for 7 min. The foam residue was extracted using the previously described procedure. The amount of foam solution was also determined to calculate the foam amount retained in samples for standard calibration purposes. The use of pig ears as foam carriers was to account for matrix effects. Each sample was analyzed by LC-MS in a SIM mode to acquire ion abundance. Ion abundance was plotted against foam concentration, corrected for actual retained foam weight, to generate external calibration curves. Unknown samples were processed similarly and their ion abundance was used to calculate the amount (in milligrams, mg) of foam per gram (g) of raw or cooked samples.

Table 4: Foam A ion abundances for external calibration

Foam A concentrations	Instrument-assigned levels	m/z 353.2 abundance	m/z 354.2 abundance
0.00%	0.00	0.00	0.00
0.16%	0.03	16661.59	4420.28
0.31%	0.06	33323.19	8840.55
0.63%	0.13	66646.38	17681.10
1.25%	0.25	133292.80	35362.21
2.50%	0.50	266585.50	70724.42
5.00%	1.00	533171.00	141448.84

Figure 5: External calibration curve for foam A

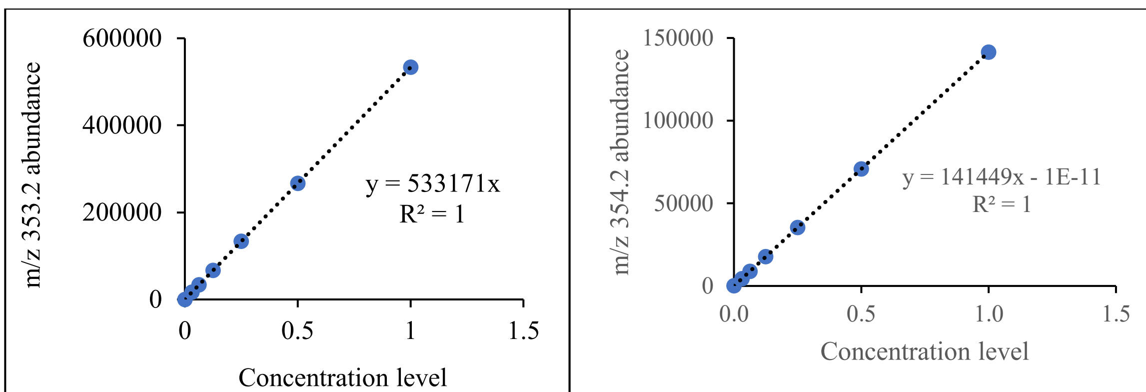


Table 5: Foam B ion abundances for external calibration

Foam B concentrations	Instrument-assigned levels	m/z 214.8 abundance	m/z 138.9 abundance
0.00%	0.00	0.00	0.00
0.50%	0.50	2911.49	3055.70
1.00%	1.00	4840.15	7239.39
1.50%	1.50	5628.29	9236.81
2.00%	2.00	6690.44	17459.76

Figure 6: External calibration curve for foam B

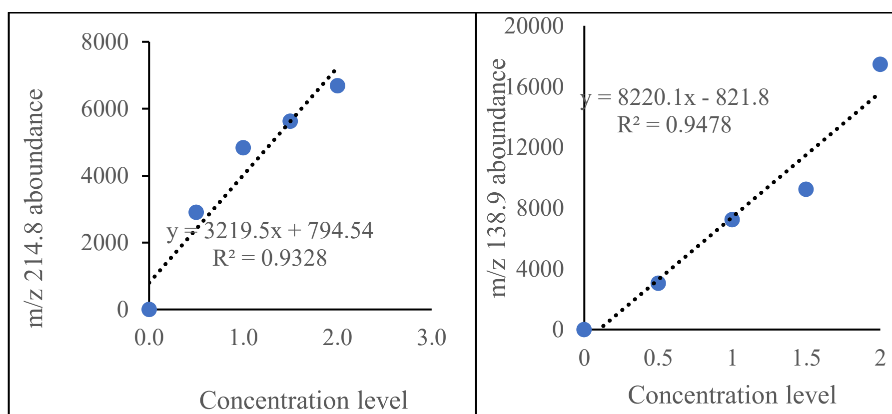
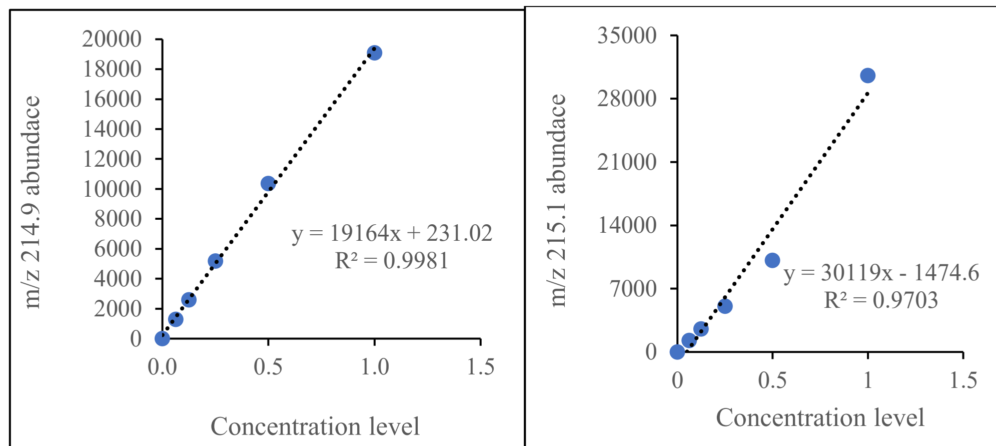


Table 6: Foam C ion abundances for external calibration

Foam B concentrations	Instrument-assigned levels	m/z 214.9 abundance	m/z 215.1 abundance
0.00%	0.00	0.00	0.00
0.06%	0.06	1294.62	1264.06
0.13%	0.13	2589.24	2528.13
0.25%	0.25	5178.48	5056.25
0.50%	0.50	10356.97	10112.50
1.00%	1.00	19096.79	30546.86

Figure 7 External calibration curve for foam C



Results:

Objective 1. To identify chemical markers of foam products used for mass depopulation via gas-filled or water-based foam.
Chemical markers were successfully identified by LC-MS that characterized and distinguished all three foams. Ions 73, 140, and 140.9 were detectable target ions for the plant-based Foam A (Figure 2, bottom panel). Ions 85, 99, 138.9, and 141 were detectable target ions for Foam B (Figure 3, bottom panel). Ions 182, 193, and 242.9 were detectable target ions for Foam C (Figure 4, bottom panel). Optimization of the MS for the target ions resulted in the identification of two ions that were highly suitable for detecting residues for each foam due to their high abundance (Table 2). Having redundancy in ions able to be used as quantitative markers increased the accuracy of detecting the foam residues on the tissues tested in Objective 2; however, for a clear presentation of the results, quantifying ion marker 354.2 was used for Foam A, marker 214.8 was used for Foam B, and 214.9 was used for calculating the amount of foam residue in mg per gram of tissue

Objective 2. To determine if any foam residues would still be present in raw and rendered pork tissues using identified chemical markers.
A total of 264 samples were tested for foam residue chemical markers by LC-MS. From three foamed carcasses per each Foam A, B, and C, triplicate samples of weighed ears, feet, snouts, and skin were tested as raw samples and cooked samples and all were analyzed with positive control water-based foam-soaked ears and negative control water-only to account for any background response and verification of methods.

After cooking tissues at conditions equivalent to rendering, foam residue chemical marker levels were reduced 3-fold, often to levels below detectable limits (reported as * indicating 0.00 mg/g of the marker ion was detected in that sample). Positive control ear sections soaked in water-based foams had detectable foam residue chemical markers at minimal levels in the raw samples that were reduced or non-detectable after cooking. Foam C had the lowest levels of foam residue chemical markers in both the raw and cooked samples. Foam A had non-detectable to negligible (0.01 mg/g or 10 ppm) levels of foam residue chemical markers after cooking. The skin from carcasses of pigs humanely killed with nitrogen gas-filled foam A had 0.16 mg/g in the raw tissue; however, after cooking skin from Foam A carcasses, the levels ranged from non-detectable (*) to 0.02 (0.01 mg/g average). Foam B samples had the highest levels of detectable foam residue chemical markers in all raw and cooked samples. Contrastingly, cooked snouts from carcasses of pigs humanely killed with nitrogen gas-filled Foam B had non-detectable levels of foam residue chemical markers whereas snouts from Foam A and C pigs had 0.01 mg/g detected in snout samples. The amounts of foam A, B, and C in the raw and cooked samples are shown in Tables 7 and 8, respectively.

Table 7. Levels of foam chemical marker ions detected in raw samples of foamed carcasses and in positive control soaked ears. Asterisks indicate non-detectable levels, i.e., levels below detectable limits of light chromatography-mass spectroscopy.

Foam	Raw Sample Type	mg/g of chemical ion marker Average	(Range)
A	***ALL***	***0.06***	***(* - 0.56)***
A	ear	0.09	(* - 0.34)
A	feet	0.01	(* - 0.03)
A	skin	0.16	(* - 0.56)
A	snout	0.03	(* - 0.12)
A	positive control soaked ear	0.02	(0.01 - 0.03)
B	***ALL***	***0.30***	***(* - 1.39)***
B	ear	0.30	(0.16 - 0.53)
B	feet	0.69	(0.19 - 1.39)
B	skin	0.06	(* - 0.11)
B	snout	0.03	(* - 0.08)
B	positive control soaked ear	0.41	(0.37 - 0.47)
C	***ALL***	***0.03***	***(* - 0.38)***
C	ear	*	(* - 0.01)
C	feet	*	(* - *)
C	skin	0.01	(* - 0.02)
C	snout	0.01	(* - 0.03)
C	positive control soaked ear	0.14	(* - 0.38)
Water (O)	***ALL***	***0.01***	***(* - 0.22)***
Water (O)	ear	*	(* - *)
Water (O)	feet	*	(* - *)
Water (O)	skin	*	(* - *)
Water (O)	snout	*	(* - *)
Water (O)	positive control soaked ear	0.03	(* - 0.22)
	ALL Samples All Foams or Water	***0.08***	***(* - 1.39)***

Table 8. Levels of foam chemical marker ions detected in cooked samples of foamed carcasses and in positive control, soaked ears. Asterisks indicate non-detectable levels, i.e., levels below detectable limits of light chromatography-mass spectroscopy.

Foam	Cooked Sample Type	mg/g of chemical ion marker Average	(Range)
A	***ALL***	***0.01***	***(* - 0.02)***
A	ear	0.01	(* - 0.01)
A	feet	*	(* - *)
A	skin	0.01	(* - 0.02)
A	snout	0.01	(* - 0.01)
A	positive control soaked ear	0.02	(0.02 - 0.02)
B	***ALL***	***0.08***	***(0.00 - 0.40)***
B	ear	0.02	(0.00 - 0.05)
B	feet	0.06	(0.05 - 0.07)
B	skin	0.03	(0.02 - 0.03)
B	snout	*	(* - *)
B	positive control soaked ear	0.29	(0.20 - 0.40)
C	***ALL***	*****	***(* - 0.01)***
C	ear	*	(* - 0.01)
C	feet	*	(* - *)
C	skin	*	(* - *)
C	snout	0.01	(* - 0.01)
C	positive control soaked ear	*	(* - 0.01)
Water (O)	***ALL***	***0.01***	***(* - 0.16)***
Water (O)	ear	*	(* - *)
Water (O)	feet	*	(* - *)
Water (O)	skin	*	(* - *)
Water (O)	snout	*	(* - *)
Water (O)	positive control soaked ear	0.03	(0.00 - 0.16)
	ALL Samples All Foams or Water	***0.02***	***(* - 0.40)***

Discussion:

Since water-based foam is used to mass depopulate poultry (AVMA 2019) in the United States (U.S.), there has been research to determine if it can be used for U.S. swine (Lorbach 2021) during emergencies (e.g., supply chain disruptions, natural disasters, disease control area stop movements). The preapproved use of firefighting foam in poultry depopulation by AVMA in combination with existing foam-generating units in the National Veterinary Stockpile, as well as their relative safety and ease of use, makes foam an attractive agent to use in local, regional, or national depopulation emergencies. Furthermore, the use of a modified rendering trailer by Lorbach et. al. sets the stage for easy carcass disposal. However, the use of foams that contain hazardous compounds such as PFAS/PFOS (aka "forever chemicals") makes it impossible to dispose of the carcasses in a manner that does not result in contamination (Mayakaduwage 2022). To that end, this research was conducted to attempt to determine if it is possible for swine carcasses that are otherwise uncontaminated (i.e., not infected with a food-borne pathogen or exposed to an adulterant) but have been covered with foam during the depopulation process to go to rendering.

This work provided much-needed information on the ability of foamed pig carcasses to be rendered. We evaluated foams for components that could serve as markers for detecting potential residues in rendered products and further determining if the rendering process eliminates the potential residues. Having found minimal to no detectable levels of Foams A (plant-based) and C (Class A/B Green-Certified firefighting foam), our results suggest that disposal options for carcasses of pigs humanely killed by water-based foam C or nitrogen gas-filled foam A or C could have rendering as a disposal option. This may not only help in the adoption of foam-based humane killing and depopulation methods in the U.S. but also provide a cost-recovery option for producers that have depopulated pigs via foam methods by allowing the carcasses to be rendered rather than composted.

While this work was conducted on a small-medium scale using tissues and parts of carcasses, rather than grinding entire carcasses and testing ground carcass samples for residues, we feel that our approach of testing the parts of the carcasses in direct contact with foam sufficiently reflects the rendering of "dead on arrivals" aka DOAs/whole carcasses. Testing whole carcasses ground into a slop/slurry or grind was neither feasible (due to lack of available two-stage grinders for research purposes only) nor necessary. Whole carcass grind testing was not necessary since our results from tissue sections of the surfaces of pigs determined that feet and snouts were the external organ tissues that retained the foam residue post-cooking. Furthermore, since our calculations of foam chemical ion markers were based on tissue weights, if one considers the dilution effect of whole body grinding, i.e., including internal organs such as skeletal muscles and bones that do not come in direct contact with the foam, it is likely that levels in all cooked tissues, even those with detectable foam in external tissues, will become negligible to non-detectable. Nevertheless, we have retained internal organ tissues (tongue, esophagus, trachea, lungs, and stomach) if needed or other research regarding foam demonstrates accumulation of foam internally. Regardless, since the majority of the cooked samples tested had no to minimally detectable chemical markers of foam residues, then procedures to prevent foam residues, such as rinsing the external surfaces of the carcasses of pigs humanely killed by foaming before grinding, could be developed and applied. At a minimum, the results of this work should help spark discussion with renderers who, historically, have been reluctant to accept foamed carcasses from pigs or poultry (Meeker, 2022)

The rendering process in the U.S. provides a method to produce products from pig carcasses routinely. In the event of an emergency, wherein large populations of pigs are depopulated, rendering facilities could also be used to dispose of animal carcasses from uninfected farms that could not be marketed through normal channels or transferred for further grow-out due to control area restrictions on pig movements (Pandey et. al. 2020). For many pathogens, the extremes of the rendering heating process (115–145°C for 40–90 minutes) are suitable for pathogen destruction and elimination (Pandey et. al. 2016), so one could argue that even infected animals could be rendered. This research was conducted at conditions that mirrored rendering, with tissues being primed with hot yellow pork grease and cooked to pathogen-killing temperatures for sufficient duration (130°C (265°F) for 75 minutes).

Regardless, if the pork industry is to support animal well-being by thoroughly evaluating and optimizing the use of foam-based methods of depopulation, the results of this research provide insight into the final step of the depopulation process – disposal. U.S. pork producers should be able to reap short-term benefits from this proposal since the results can be shared with the rendering industry, an allied industry that is committed to decreasing animal waste and allowing foamed carcasses to be removed from farms for rendering. The potential long-term benefits are decreasing land use by eliminating the need for compost piles on or around pig farms as well as the aforementioned opportunity cost recovery.

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