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2900 100th Street, Suite 309, Des Moines, IA 50322-3855, USA

John B. Luchansky,[1*] Bradley A. Shoyer,[1] Laura E. Shane,[1] Manuela Osoria,[1] Stephen G. Campano,[2] and Anna C.S. Porto-Fett[1]

[1]U.S. Dept. of Agriculture, Agricultural Research Service, 600 East Mermaid Lane, Wyndmoor, PA 19038, USA
[2]Hawkins, Inc., 2381 Rosegate, Roseville, MN 55113, USA

Inactivation of *Listeria monocytogenes* and Shiga Toxin-Producing *Escherichia coli* in "Soupie," a Homemade Soppressata

ABSTRACT

Viability of *Listeria monocytogenes* and Shiga toxin-producing *Escherichia coli* (STEC) was monitored in "soupie," a homemade soppressata. Coarse-ground fresh ham was mixed with nonmeat ingredients, a starter culture (ca. 6.0 log CFU/g), and one pathogen cocktail (ca. 6.5 log CFU/g). The batter was then fine ground, stuffed into fibrous casings, and fermented at 26.7°C and ca. 90 ± 5% relative humidity (RH) for ≤ 48 h to achieve a pH of ≤ 5.3. Chubs were dried at 15.6°C and ca. 87 ± 5% RH for 5 days, flattened under weights for 3 days, and then dried for an additional 21 days at 4°C and ca. 73 ± 5% RH. Half of the chubs were vacuum sealed individually in bags with 8 mL of sunflower oil, and the other half were submerged in sunflower oil (ca. 1.5 L) within covered plastic containers; all chubs were stored for 6 months at 20°C. Fermentation and drying delivered a ≤ 1.2-log reduction in levels of both pathogens. Regardless of storage conditions, a ≥ 5.0-log reduction was observed within 1 and 4 months of storage at 20°C for STEC and *L. monocytogenes*, respectively. These data establish that artisanal soupie, prepared and stored as described here, does not provide a favorable environment for pathogen persistence or proliferation.

INTRODUCTION

Immigrants from Eastern and Southern Europe entering the United States in the late 1800s and early 1900s brought with them many ethnic traditions and practices, with music, religion, and specialty foods among the most notable *(9, 41)*. A prime example is the preponderance of immigrants from the Calabria region of Italy who settled in the coal regions of Northeastern Pennsylvania (NEPA) *(41)* and who continue to make an artisanal soppressata (also known as "soupie" or "soupy") *(40)*. Although familial recipes and processes may differ somewhat, longstanding traditions call for locally sourced pork and a handful of nonmeat ingredients that are ground, mixed, (hand) stuffed into beef middle natural casings, fermented, flattened, dried, and then stored under oil for months to years at ambient temperature *(35)*. Each new year, families gather to make soupie for consumption in the year ahead as a portable snack or as a special ingredient

*Author for correspondence: Phone: +1 215.233.6676; Fax: +1 215.233.6406; Email: john.luchansky@usda.gov

in various dishes, such as omelets, macaroni and cheese, and spaghetti, and for use as a topping on pizza, a component of antipasto, or an item on a charcuterie board *(7, 23)*. For the coal miners of NEPA, soupie was also the handheld and shelf-stable snack consumed while at work. Soupie is now available for purchase from commercial processors and a handful of specialty butcher shops, and several food markets in NEPA also sell the requisite pork, spice mix, and casings so that denizens of NEPA can make soupie at home *(7, 23)*. For these reasons, additional research is warranted to improve both the wholesomeness (i.e., control pathogens) and quality of soupie (i.e., extend shelf life, limit sensory defects such as discoloration, and maintain nutritional attributes).

Like other dry-cured fermented sausage, soupie is not a primary vehicle of foodborne illness nor is it a food typically responsible for product recalls. Our review of the available literature did not identify recalls or outbreaks directly attributed to soupie contaminated with foodborne pathogens. However, pathogens such as *Listeria monocytogenes*, Shiga toxin-producing *Escherichia coli* (STEC), and *Salmonella* have been isolated from fermented sausage, and several outbreaks and recalls have been associated with fermented sausage attributed to these bacterial pathogens *(10–14, 20, 26, 29, 32, 44, 46, 47)*. In 2009, a nationwide *Salmonella* Montevideo outbreak was linked to the (imported) ground red and black pepper used to coat the incriminated salami products *(12)*. This outbreak is germane to the present study because ground pepper is a common ingredient in soupie recipes *(35, 40)*. Regarding STEC, in 2011, a recall of ca. 23,000 lb (10,442 kg) of Lebanon bologna, a fermented semidry sausage, was issued due to contamination with *E. coli* O157:H7 *(44)*. This incident resulted in 14 illnesses across five states; three persons required hospitalization but did not present with hemolytic uremic syndrome, and no deaths were reported *(13)*. Regarding *L. monocytogenes*, in 2019, ca. 25,000 lb (11,350 kg) of ready-to-eat sausage, such as sliced sausage for pizza and sliced or chopped pepperoni, were recalled due to possible adulteration with *L. monocytogenes (47)*. As another example, a 2020 multistate outbreak of listeriosis attributed to Italian-style salami, mortadella, and prosciutto resulted in 12 hospitalizations and one death *(14)*. Depending on the strains, product formulation and type, and processing and storage conditions, both STEC and *L. monocytogenes* can survive fermentation and/or drying of various fermented sausage *(5, 17, 19, 21, 22, 27, 34)*. The persistence of these pathogens in fermented sausage raises public health concerns because, as previously reported by our group and other investigators, in general, fermentation alone delivers only a ≤ 2.0-log reduction of STEC and *L. monocytogenes (21, 22, 30)*. To deliver the 2.0- or 5.0-log reductions of STEC, *Salmonella*, or *L. monocytogenes (36, 37, 42, 45)* required for some fermented sausages, evaluations are needed for (i) ingredients that impart both functionality and antimicrobial properties, (ii) bacteriocinogenic starter cultures with antagonism toward regulated pathogens, and/or (iii) postfermentation interventions (e.g., heat or high pressure and extended storage under controlled conditions). Given the absence of survey data quantifying the presence, levels, or types of regulated pathogens in soupie coupled with the absence of data on the fate of pathogens that may on occasion become associated with this hand-crafted soppressata salumi, studies are needed to allay or correct any perceived or actual food safety concerns for soupie.

From a public health perspective, soupie has not been associated with product recalls or human illnesses, most likely because it is largely produced on a comparatively small scale within very limited geographic regions of NEPA by consumers who have little interest in, experience with, or opportunities for process verification or product surveillance. However, soupie could harbor pathogenic microbes because (i) it is commonly made by consumers in the home using locally sourced (raw) ingredients with only moderate consistency and limited control over key processing parameters (e.g., temperature, air flow, and relative humidity [RH]), (ii) home fabricators may rely on the indigenous flora to initiate or conduct the fermentation (i.e., not a commercial starter culture), and (iii) it is handled extensively and is typically stored for an extended time period. Therefore, soupie is an excellent model system, and data related to its safety and quality attributes can also provide important insights into the wholesomeness of other comparably produced specialty (fermented) meats.

Both bacterial and parasitic (e.g., *Trichinella spiralis*) pathogens can compromise the safety of fermented pork products, including soppressata and Italian-style salami, when such products are not properly prepared, handled, or stored *(20, 26, 29, 32, 44, 46, 47)*. Given the absence of scientific data related to pathogen presence or load or process validation and that pathogens such as those listed above may be present and possibly persist in dry-cured salami, for this initial study we separately monitored the fate of a multistrain cocktail of gram-positive (*L. monocytogenes*) and gram-negative (STEC) bacteria during the preparation of soupie.

MATERIALS AND METHODS

Bacterial strains

The following eight-strain cocktail of genetically marked strains of STEC was used to inoculate raw batter for preparing soupie: (i) H30 (serotype O26:H11, isolate from an infant with diarrhea), (ii) JB1-95 (serotype O111:H–, clinical isolate), (iii) CDC 96-3285 (serotype O45:H2, human stool isolate), (iv) CDC 90-3128 (serotype O103:H2, human stool isolate), (v) ATCC BAA-2326 (serotype O104:H4, human stool isolate), (vi) CDC 97-3068 (serotype O121:H19, human stool isolate), (vii) 83-75 (serotype O145:NM, human stool isolate), and (viii) USDA-FSIS 011-82 (serotype O157:H7, meat isolate). The following five-strain cocktail of genetically marked strains of *L. monocytogenes* also was used to inoculate raw batter for preparing soupie: (i)

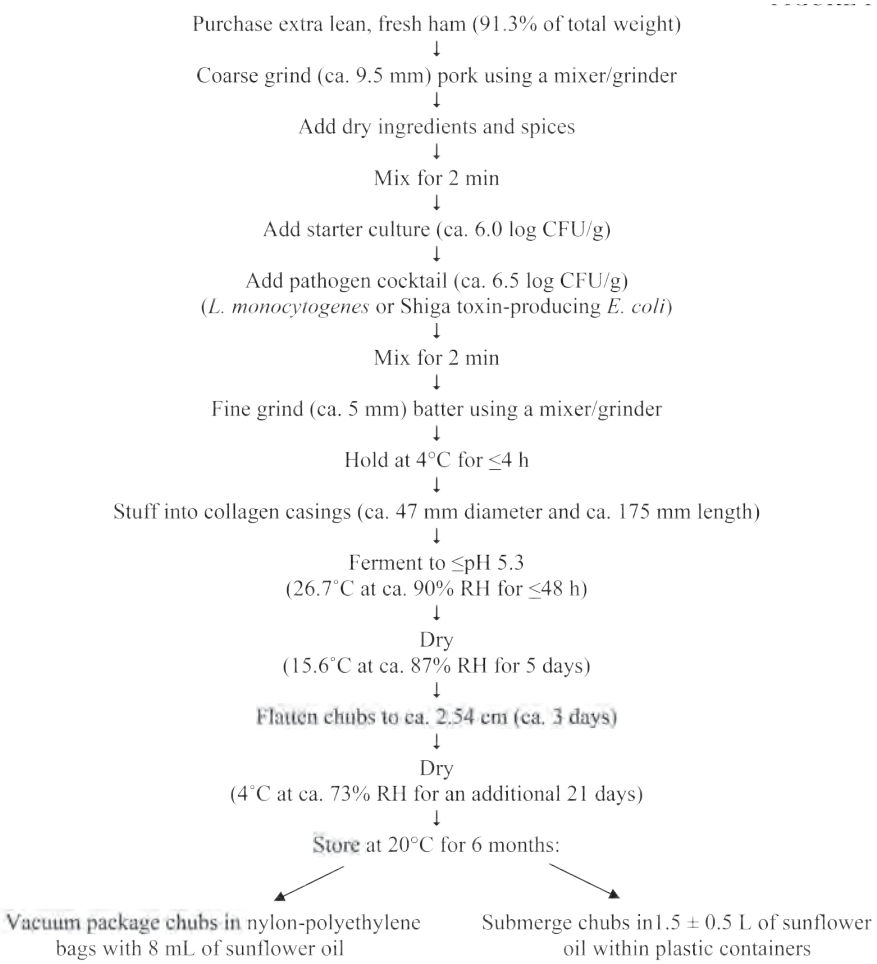

Figure 1. Flowchart describing manufacture and storage of soupie.

Scott A (serotype 4b, clinical isolate), (ii) H7776 (serotype 4b, frankfurter isolate), (iii) LM-101M (serotype 4b, beef and pork sausage isolate), (iv) F6854 (serotype 1/2a, turkey frankfurter isolate), and (v) MFS-2 (serotype 1/2c, environmental isolate from a pork processing plant). Strains in each cocktail were maintained and utilized as previously described *(25, 33)*. Each cocktail was separately prepared by transferring a loopful of an isolated colony of each strain of STEC or *L. monocytogenes* to separate tubes containing 10 mL of brain heart infusion (BHI) broth (Difco, BD, Franklin Lakes, NJ) that were subsequently incubated for ca. 18 ± 2 h at 37°C. From each tube, 0.5 mL was separately transferred into a flask containing 50 mL of BHI broth and incubated for an additional ca. 18 ± 2 h at 37°C. The entire volume (50 mL) of each tube of each of the freshly grown eight strains of STEC or five strains of *L. monocytogenes* were then combined within a sterile flask that was held at 4°C for about 30 min *(15, 22)*.

Inoculation, fermentation, and storage of soupie

Use of an internet search engine yielded a handful of (very similar) recipes for soupie that were used by inhabitants of NEPA, from which a generic formulation and assembly protocol for soupie was derived *(Fig. 1)*. This old-world-stye soppressata (soupie) batter was prepared in six separate batches (ca. 22.7 kg per batch; three batches for each pathogen cocktail) using coarse ground (9.5-mm grinding plate) extra lean fresh ham (20.8 kg, 91.3% of total weight) obtained from a local butcher (Rieker's Prime Meats, Philadelphia, PA). Before fermentation, the following ingredients and spices were added to the ground pork: red wine (5%; Cabernet Sauvignon, Franzia, Livermore, CA), pure ocean sea salt (2.5%; SaltWorks, Woodinville, WA), paprika (0.38%; The Sausage Maker, Buffalo, NY), Insta Cure #2 (0.24%; The Sausage Maker), cayenne pepper (0.22%; The Sausage Maker), powdered dextrose (0.2%; The Sausage Maker), and black pepper (0.08%; The Sausage Maker). The pork and spices were mixed for about 2 min (model 4346, Hobart Corp., Troy, OH), and 100 mL of starter culture (5.68 g in 100 mL of sterile tap water, target concentration of ca. 6.0 log CFU/g of batter; Bactoferm LC, Chr. Hansen, Hørsholm, Denmark) and 227 mL of the freshly grown *L. monocytogenes* or STEC cocktails (target concentration ca. 6.5 log CFU/g

of batter) were added to separate batches of batter. Mixing continued for an additional 2 min, and the inoculated batter was then finely ground through a 5-mm grinding plate (model 4346; Hobart) into high-density polyethylene containers (21 by 12 by 8.75 in. [53 by 30.5 by 22 cm]; ToteAll 2000, Bunzl/Koch Equipment, Kansas City, MO) lined with polyethylene bags (PolyTote Liner, Bunzl/Koch). After holding at 4°C for up to 4 h, the batter was loaded into a hydraulic-piston stuffer (model SC-50, Koch Equipment) and stuffed into pretied 47-mm-diameter collagen casings (The Sausage Maker) to a length of ca. 17.5 cm. The resulting chubs were hand tied with twine (butcher's twine, The Sausage Maker) and hung vertically in a temperature- and humidity-controlled walk-in environmental chamber (model AB-3-F/W-0-RZ-HV-DX-EL-UL, Thermmax Scientific, Warminster, PA). Based on information collected from the literature and via discussions with inhabitants of NEPA who prepare soupie at home, chubs were fermented at 26.7°C and ca. 90 ± 5% RH for up to 48 h to achieve pH of ≤ 5.3.

After fermentation, the conditions of the environmental chamber were changed to dry the chubs at 15.6°C and ca. 87 ± 5% RH for 5 days. The chubs were then placed side by side in a single layer within high-density polyethylene containers lined with polyethylene bags, and the excess liner was folded over to cover the chubs. These containers were then nested four or five high, and a ca. 10-kg weight was placed in the topmost (empty) container to flatten the chubs to a thickness of ca. 2.54 cm (≤ 3 days). The flattened chubs were further dried for an additional 21 days at 4°C and ca. 73 ± 5% RH, after which the twine and casings were removed with alcohol-sterilized scissors. Half the chubs were placed individually into sterile nylon-polyethylene bags (Prime Source Packaging, Houston, TX) with 8 mL of sunflower oil (non-GMO sunflower oil, Catania Oils, Ayer, MA) and vacuum sealed to 950 mBar (Ultravac-500, Bunzl/Koch), and the other half of the chubs were placed into several plastic containers (9 or 10 chubs per container) with lids (18 by 12 by 12 in. [45.7 by 30.5 by 30.5 cm]; model S14599, Uline, Pleasant Prairie, WI) containing 1.5 ± 0.5 L of sunflower oil to completely submerge the chubs. Because most of the soupie made by the consumers and small retailers we met in NEPA was stored at room temperature and because 20 ± 2°C is typical for room (indoor) temperature *(1)*, both sets of chubs were stored at 20°C for 6 months (most consumers and retailers can maintain an indoor temperature of 20 ± 2°C without undue hardship or expense). At preselected sampling times during this entire process, portions of the meat, batter, or chubs were analyzed for water activity (a_w), pH, and pathogen presence and levels.

Microbiological and chemical analyses of inoculated soupie

At various times during storage, soupie chubs submerged in oil in containers were lifted from the container with alcohol-sterilized stainless steel tongs. After allowing excess oil to drip back into the container, chubs were placed individually onto Styrofoam trays (1012S, Genpak, Glen Falls, NY) for sampling. At the same times, bags of vacuum-packaged each chub were opened with alcohol-sterilized scissors, and chubs were removed with alcohol-sterilized tongs and placed individually onto Styrofoam trays for sampling. Pathogens were enumerated from soupie by transferring a 25-g portion of each chub into filter bags (type XX-C003, Microbiology International, Frederick, MD) containing 25 mL of sterile 0.1% peptone water (Difco, BD) and macerating for 2 min in a stomacher (Stomacher 400, Seward, Cincinnati, OH). Next, 100 to 500 µL of the resulting filtrate were surface plated in duplicate, with and without dilution in 0.1% sterile peptone water, onto modified Oxford agar (MOX; Difco, BD) or sorbitol MacConkey agar (SMAC; Difco, BD) plates containing rifampin (100 µg/mL; TCI America, Portland, OR) to recover *L. monocytogenes* and STEC, respectively. Plates were incubated at 37°C for 24 h (SMAC) or 48 h (MOX), and colonies typical for each pathogen were enumerated and levels were expressed as log CFU per gram. Although some injured cells of STEC or *L. monocytogenes* may not be recovered on SMAC or MOX, respectively, given the presumed harshness of these recovery media for stressed cells, when pathogen levels decreased to below the detection limit (ca. ≤ 0.26 log CFU/g) by direct plating, the samples were enriched as described previously *(15, 22)* to assess for injured and very low levels of the target pathogens. For STEC, 1 mL of the resulting filtrate was transferred into 9 mL of EC broth plus novobiocin (20 µg/L; Difco, BD) and incubated at 37°C for 24 h. A portion of EC broth culture was then swabbed onto SMAC plus rifampin agar plates with a cotton-tipped swab. Plates were then incubated at 37°C for 24 h *(22)*. For *L. monocytogenes*, 1 mL of the resulting filtrate was transferred into 9 mL of University of Vermont medium broth (UVM; Difco, BD) and incubated at 37°C for 24 h. Following incubation, 0.1 mL of the UVM culture was transferred into 9.9 mL of Fraser broth (Difco, BD) and incubated at 37°C for 24 h. The Fraser broth culture was then streaked onto MOX plus rifampin agar plates with a cotton-tipped swab, and plates were incubated at 37°C for 24 h *(15)*. Following incubation, SMAC and MOX plates were analyzed for the presence or absence of surviving cells of STEC or *L. monocytogenes*, respectively.

The pH and a_w of soupie were determined as described elsewhere *(34)*. At each sampling point, the a_w of each of three chubs was separately measured by placing ca. 3 g of soupie into an electronic a_w meter (Aqualab model series 3, Decagon Devices, Pullman, WA) according to the manufacturer's instructions. For pH measurements, a 25-g portion from each of the same three chubs used for a_w determinations was separately transferred to a filtered stomacher bag containing 25 mL of 0.1% peptone water and then macerated for 2 min. The pH of the resulting slurry was measured (model 6000P pH/temperature electrode and model 5500 pH meter, Daigger, Vernon Hills, IL) as described *(34)*. The matrix for

this study consisted of three trials and three replicates for each sampling interval, for a total of nine samples of soupie tested at each sampling time. In each of three trials for each processing step and storage day, three soupie chubs were analyzed for the presence and levels of either STEC or *L. monocytogenes* and for pH and a_w ($N = 3$, $n = 3$).

Proximate composition analyses of purchased soupie

Soupie was obtained from one online vendor and six retail markets or delis in NEPA. Because of the proprietary nature of the formulation and protocols practiced by each processor, it was not possible to obtain specific information on how each brand of soupie purchased at retail establishments was prepared, aged, or stored. All soupie samples from retail markets were purchased on 2 days about 2 months apart. The soupie from one establishment was sold as slices within closed plastic containers without any added oil, soupie from five vendors was sold as chubs submerged in oil, and the seventh brand was sold vacuum packaged in a nominal volume of oil. After allowing residual oil to drip off, each chub was wrapped in butcher paper and placed in a sealable plastic bag by a store employee. Upon arrival at our laboratory, the soupie was removed aseptically from the original package with alcohol-sterilized tongs. Portions (ca. 360 to 800 g) from multiple chubs or multiple containers of slices from each type or brand were aseptically and separately pooled and then placed into sterile nylon-polyethylene bags that were heat sealed under ca. 10% vacuum. Products were stored refrigerated for up to 8 days and then analyzed by a commercial laboratory using the following AOAC approved methods *(4)* for fat (AOAC 960.39), salt (AOAC 983.14), ash (AOAC 920.153), moisture (AOAC 950.46Bb), protein (AOAC 991.20.i), sodium nitrite (AOAC 973.31), titratable acidity (AOAC 942.15), a_w (AOAC 978.18), and carbohydrates by calculation. Products also were analyzed by the same commercial laboratory using approved methods *(4)* for the presence of *Clostridium perfringens* (AOAC 976.30), STEC (serogroups O26, O45, O103, O111, O121, and O145 [AOAC RI 091301] and O157:H7 [AOAC RI 031002]), *L. monocytogenes* (AOAC 2003.12), coagulase-positive *Staphylococcus* (AOAC 975.55), and *Salmonella* (AOAC 2003.09).

Statistical analyses

Means and standard deviations were calculated from data for each trial using Excel 2010 software (Microsoft, Redmond, WA). Observed reductions in STEC and *L. monocytogenes* levels in soupie stored under oil or under vacuum across sampling points were characterized using Brain-Cousens sigmoidal regression models *(39)*. These regression models were specified to obtain estimates of the time required to achieve pathogen reductions to 25% of the initial pathogen levels by using the RD_k ($k = 75$) parameterization of the Brain-Cousens model *(39)*.

RESULTS AND DISCUSSION

Survey of soupie available for purchase

For over a century soupie has been prepared across NEPA by families using recipes passed down from one generation to the next. What is missing, however, are data on the overall safety and quality attributes of soupie, including its chemical composition and the presence and levels of bacterial pathogens and their potential ability to persist and proliferate in the product. For these reasons, we conducted a small-scale, informal, and nonrandomized survey of soupie available for purchase in NEPA to gain insight into the availability, characteristics, and chemical and microbiological profiles of this handheld, protein-dense artisanal snack *(Fig. 2)*. Of the seven brands collected (one to four chubs and/or containers of each brand), three brands had safe-handling information on the label. Although the other four brands lacked safe-handling instructions, they had ingredient information on the label, such as pork, salt, pepper, and/or sodium nitrate and sodium nitrite. Six brands were sold as chubs of 160 to 300 g each, with average dimensions of ca. 19.4 cm long by 5.1 cm wide by 2.2 cm thick. For the single brand sold as slices in a plastic container (ca. 58 g per container), soupie was sliced to dimensions of 6.4 cm wide by 1.3 cm high by 0.25 cm thick. Chubs of five brands were stored fully submerged in oil, those of one brand were vacuum packaged with a nominal amount of oil, and those of another brand were sliced and sold in plastic containers without oil; prices ranged from ca. $19 to $39 per pound. Among the seven brands surveyed, differences in chemical composition among brands *(Table 1)* were expected and may be attributed, at least in part, to the presumed differences in how soupie was fermented and dried (i.e., temperature and RH), the inclusion or not of starter cultures, and/or the formulation (i.e., levels of salt and sugar) of each brand tested. Regardless of these differences, and although the retail soupie surveyed here displayed a higher final pH (mean, ca. 5.7 ± 0.4) than many other fermented meats, the combined effect of a relatively low a_w (0.830 ± 0.030 after drying), relatively high salt concentration (5.2 ± 0.8%), and presence of residual nitrite (mean, < 5.0 ppm) would presumably be sufficient to inhibit the growth of pathogenic bacteria such as *L. monocytogenes*, *Salmonella*, STEC, and *Clostridium* spp. Microbiological analyses of the retail soupie purchased for this study revealed the absence (in 25 g) of *Salmonella*, the "big six" STEC (serogroups O26, O45, O103, O111, O121, and O145), *E. coli* O157:H7, and *L. monocytogenes*. However, all brands tested were positive for coagulase-positive *Staphylococcus* (< 10 CFU/g) and *C. perfringens* (< 10 to < 100 CFU/g).

Viability of pathogens inoculated into soupie

No significant differences ($P > 0.05$) in viability of STEC or *L. monocytogenes* were observed following fermentation between soupie that was vacuum packaged with oil and soupie that was submerged in oil during extended storage at 20°C.

Figure 2. Photographs of soupie purchased at retail: A, chubs stored under oil; B, chubs stored under vacuum; C, chubs removed from oil; D, interior of soupie.

TABLE 1. Proximate composition of soupie purchased at food markets

Analytes	Brands							Average ± SD[a]
	MtC-Br	Sham-An	Sham-Jo	MtC-Vi	Chen-Lu	Min-To	Coal-Iris	All Brands
Fat (%)	38.0	8.92	12.8	9.9	28.1	14.0	9.26	17.28 ± 11.29
Carbohydrates (%)	1.66	1.95	2.67	2.32	1.20	0.74	1.55	1.73 ± 0.66
Protein (%)	29.29	42.0	39.43	41.43	32.70	41.45	43.05	38.48 ± 5.31
Moisture (%)	25.58	39.74	35.77	39.45	32.21	37.36	37.69	35.4 ± 5.02
Ash (%)	5.51	7.42	9.33	6.90	5.84	6.44	8.45	7.13 ± 1.38
Salt (%)	4.17	5.31	6.22	5.43	4.38	4.64	6.0	5.16 ± 0.79
Acidity (as lactic acid)	2.61	2.79	3.27	6.28	4.54	2.81	3.3	3.66 ± 1.32
Nitrite (ppm)	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0 ± 0.0
pH	4.97	5.96	5.84	6.27	5.64	5.68	5.42	5.68 ± 0.41
Water activity	0.808	0.864	0.804	0.827	0.828	0.867	0.834	0.83 ± 0.03

[a]Standard deviation.

However, a notable difference ($P < 0.05$) was observed in the time required to achieve a ≥ 5.0-log reduction of the pathogens during storage; *L. monocytogenes* was less responsive to treatment than was STEC *(Fig. 3)*. Fermentation alone resulted in only a ≤ 0.3-log reduction in the target pathogens. No further reductions of any significance (ca. a ≤ 0.9-log reduction) were noted during subsequent drying of the flattened soupie chubs. Regardless of whether soupie were vacuum packaged or submerged in oil, levels of STEC and *L. monocytogenes* decreased to below the level of detection (≤ 0.26 log CFU/g) after 1 and 4 months, respectively, of storage at 20°C. Such reductions were likely achieved by the integrated lethality of multiple stresses on pathogen viability, including pH, a_w, time, RH, and temperature. STEC cells were recovered only via enrichment culture from most soupie (61.1%; 11 of 18 samples) that were either vacuum packaged or submerged in oil after 2 months of storage at 20°C; no STEC cells were recovered from soupie stored > 3 months at 20°C (data not shown). Regardless of whether soupie were vacuum packaged with oil or submerged in oil during storage, *L. monocytogenes* cells were recovered from soupie only by enrichment culture (5.6%; 1 of 18 samples) within 5 months at 20°C; no *L. monocytogenes* cells were recovered at 6 months of storage (data not shown). Processors must validate that the formulation and fabrication protocol for fermented sausages such as soupie deliver the requisite reduction of undesirable microorganisms and must continually verify their hazard analysis and critical control point plans and adhere to both good manufacturing practices and sanitation standard operating procedure programs to ensure the microbial safety of their products *(2)*.

Intrinsic properties of inoculated soupie

Although endemic to small coal towns across NEPA for decades, soupie is not listed in the USDA *Food Standards and Labeling Policy Book (43)* or 9 CFR Part 319 ("Definitions and standards of identity or composition") *(48)* and, thus, is lacking a standard of identity. For the purposes of the present study, it may (arguably) be appropriate to take the liberty of comparing the intrinsic attributes of soupie with those of soppressata because soupie is referred to both in the literature and in the marketplace as a homemade soppressata-type, dry-fermented and cured sausage that shares common ingredients and processing conditions with traditional soppressata *(35, 40)*. As a dry-fermented sausage, the typical moisture-to-protein ratio of soppressata would be ≤ 1.9:1 and, as determined by regional preferences, would include paprika, garlic, and/or red peppers *(43)*. The shelf stability of a dry salami such as soppressata is dependent on a sufficient decrease in both pH (i.e., pH ≤ 5.3 after fermentation) and a_w (i.e., a_w ≤ 0.91 after drying) to eliminate or inhibit outgrowth of foodborne pathogens during storage *(6)*. A review of the available literature revealed that the final pH for dry sausage may range from 5.2 to 5.8, and the final a_w may range from 0.91 to 0.85 *(49)*. The pH at the end of fermentation (5.2) and the a_w at the end of drying and storage (≤ 0.87) attained by the products prepared and evaluated in this study are within the acceptable range for a shelf-stable product *(49) (Table 2)*. With these low pH and a_w values, a ≥ 5.0-log reduction of STEC and *L. monocytogenes* was achieved, validating that the process and recipe for soupie evaluated in the present study resulted in a final product that was both safe and shelf stable. Regardless of the pathogen tested, the pH of the batter decreased from ca. 5.8 to ca. 5.2 after fermentation. The pH after 21 days of drying increased to ca. 5.7 and then increased further to ca. 6.3 after 6 months of storage. Similar increases in the pH of fermented sausage during drying and/or storage (e.g., from 4.9 to 5.3 after fermentation and then increasing to 6.0 to 6.6 after drying) have been reported by others *(8, 16, 24)*. As summarized for other fermented products *(18, 38)*, the observed increase in the pH of soupie from fermentation through drying and storage may have been due to (i) complete utilization of the dextrose added to the formulation as part of the starter culture and subsequent utilization of available proteins as an energy source, (ii) formation of ammonia as a consequence of chemical transformations of free amino acids, (iii) an increase in buffering compounds related to degradation of meat proteins, and/or (iv) a decrease in dissociation of electrolytes with a lowering of the pH during fermentation. Another possible explanation for the observed increase in pH from fermentation to drying and storage of soupie is the high initial protein content resulting from the use of extra-lean pork (i.e., an isoelectric point for muscle proteins of ca. pH 5.4) and the generation of lactic acid (i.e., a weak acid) during fermentation (ca. pH 5.2). Meat proteins are strong buffers, and the soupie prepared in this study had a high initial protein content and relatively high initial moisture content. These attributes coupled with the relatively low dissociation constant of lactic acid (pKa 3.8) make it likely that the lactic acid accumulated as a result of fermentation would eventually dissociate due to moisture loss during drying and storage and convert to lactate, which, in turn, would result in an increase in pH, as observed. The a_w of the soupie decreased from ca. 0.973 after fermentation to ca. 0.870 after drying. After storage for 6 months at 20°C either under vacuum in the presence of oil or submerged in oil, the final a_w of soupie averaged ca. 0.844. The decrease of a_w, alone or in combination with other intrinsic factors, during fermentation and drying of a dry-cured sausage is crucial for the shelf stability of these products. For example, intrinsic parameters such as pH ≤ 5.2 and a_w ≤ 0.95, only pH ≤ 5.0, or only a_w ≤ 0.91 are essential for the microbial shelf stability of dry sausage, presuming that other hurdles such as salt and nitrates or nitrites are within the concentrations required for dried or cured sausage *(3)*. Although the soupie prepared in this study achieved ca. pH 5.7 after drying, it would be deemed shelf stable because of its a_w of ≤ 0.877 after drying *(Table 2)*. The final a_w of soupie decreased to ca. 0.840 after storage for 6 months at 20°C.

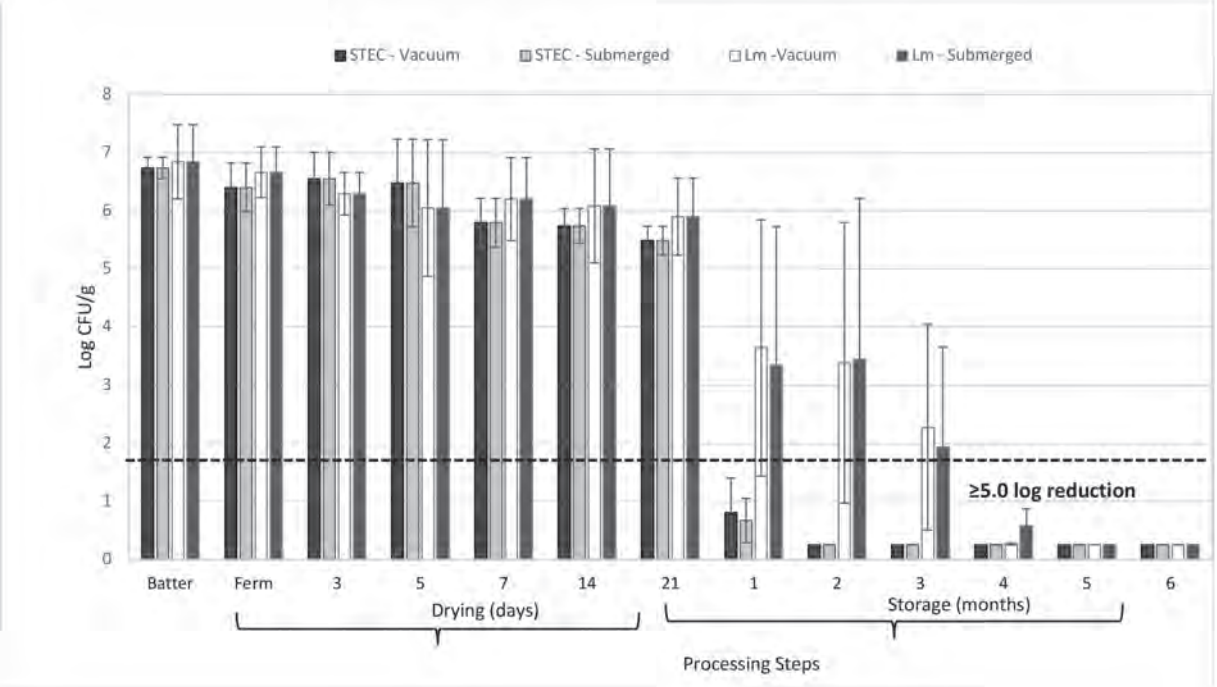

Figure 3. Recovery of cells (log CFU/g) of Shiga toxin-producing *Escherichia coli* (STEC) and *Listeria monocytogenes* (Lm) from soupie during fermentation, drying, and storage under vacuum in oil and submerged in oil in plastic containers. The error bars represent the standard deviation of the mean (N = 3, n = 3).

TABLE 2. Water activity and pH of soupie during fermentation, drying, and storage (N = 3, n = 3)

Processing Steps	Water Activity				pH			
	STEC		*L. monocytogenes*		STEC		*L. monocytogenes*	
Batter	0.973 ± 0.006		0.969 ± 0.007		5.83 ± 0.11		5.79 ± 0.10	
Fermentation	0.973 ± 0.002		0.973 ± 0.002		5.20 ± 0.09		5.26 ± 0.17	
Drying								
Day 3	0.969 ± 0.0		0.967 ± 0.004		5.59 ± 0.01		5.36 ± 0.29	
Day 5	0.969 ± 0.005		0.965 ± 0.006		5.34 ± 0.26		5.55 ± 0.48	
Day 7	0.942 ± 0.009		0.945 ± 0.010		5.71 ± 0.24		5.59 ± 0.37	
Day 14	0.907 ± 0.012		0.912 ± 0.014		5.80 ± 0.25		5.68 ± 0.29	
Day 21	0.869 ± 0.016		0.877 ± 0.012		5.75 ± 0.23		5.69 ± 0.26	
Storage	Vacuum	Submerged	Vacuum	Submerged	Vacuum	Submerged	Vacuum	Submerged
Month 1	0.874 ± 0.020	0.872 ± 0.017	0.874 ± 0.019	0.876 ± 0.022	5.92 ± 0.24	5.98 ± 0.27	5.78 ± 0.31	5.85 ± 0.34
Month 2	0.856 ± 0.017	0.864 ± 0.019	0.869 ± 0.023	0.864 ± 0.021	6.03 ± 0.29	5.96 ± 0.20	5.89 ± 0.21	5.95 ± 0.22
Month 3	0.844 ± 0.052	0.856 ± 0.024	0.862 ± 0.022	0.854 ± 0.024	6.12 ± 0.18	6.03 ± 0.17	5.97 ± 0.20	5.98 ± 0.21
Month 4	0.843 ± 0.026	0.846 ± 0.034	0.854 ± 0.025	0.842 ± 0.024	6.11 ± 0.07	6.21 ± 0.10	6.02 ± 0.06	6.18 ± 0.17
Month 5	0.855 ± 0.025	0.854 ± 0.023	0.856 ± 0.020	0.854 ± 0.026	6.15 ± 0.08	6.28 ± 0.15	6.10 ± 0.08	6.32 ± 0.20
Month 6	0.844 ± 0.020	0.848 ± 0.019	0.848 ± 0.022	0.837 ± 0.014	6.26 ± 0.13	6.34 ± 0.26	6.24 ± 0.07	6.37 ± 0.20

Although storage of soupie under vacuum in the presence of oil or submersion of soupie under oil in plastic containers resulted in reduced levels of both STEC and *L. monocytogenes*, the absence of oxygen may also have provided a favorable environment for persistence or proliferation of anaerobic pathogens such as *Clostridium* spp. Outbreaks of botulism have been attributed to consumption of vegetables contaminated naturally with *Clostridium botulinum* and stored in oil, presumably because such products were not properly prepared or handled *(28, 31)*. Because the soupie purchased at retail for the present study harbored low levels of *C. perfringens* (<10 to 100 CFU/g) and based on the observed intrinsic characteristics of this soupie *(Table 1)*, additional studies may be warranted to monitor the fate of *Clostridium* spp. during manufacture and storage of soupie to fully ascertain the potential for any public health concerns. Although the retail soupie tested here also contained low levels (<10 CFU/g) of coagulase-positive *Staphylococcus*, a cursory review of the available literature did not reveal any recalls or illnesses caused by staphylococcal contamination of foods stored under oil. The combined effects of pH, a_w, salt, and atmosphere and the fact that in general staphylococci are poor competitors in foods likely contributed to the absence of staphylococcal food poisoning caused by products stored under oil.

Concluding remarks

The citizenry of the towns and villages in the coal regions of NEPA take much pride in their rich history and attendant culinary creations. At present, soupie is one of only a handful of specialty or ethnic foods that remain from the halcyon days when mining of anthracite coal was at its heyday across NEPA. Although soupie has been made for over a century across NEPA without a documented incident, its steadfast popularity, absence of a standard of identity, in-home preparation, and the absence of any quantitative data on its wholesomeness or intrinsic properties provided justification for collecting general information on its availability and types and for generating scientifically sound data on its safety and quality. For the soupie purchased at retail for the present study, the pH and unbound available moisture ranged from ca. pH 5.0 to 6.3 and ca. a_w 0.80 to 0.87, respectively *(Table 1)*. For the inoculated soupie prepared for this study, the pH and unbound available water after drying were ca. pH 5.7 and ca. a_w 0.877, respectively *(Table 2)*. These pH and a_w values combination were sufficiently antagonistic to the inoculated pathogens. As a result, the recipe and protocol used to prepare soupie in this study resulted in a ≥ 5.0-log reduction of STEC and *L. monocytogenes* after storage at 20°C for 1 and 4 months, respectively. Thus, when properly prepared, stored, and handled, soupie would pose a minimal risk to public health even if low levels of either STEC or *L. monocytogenes* were occasionally associated with this product. Validation of the safety of a recipe and fabrication protocol for soupie as detailed in this study will contribute to the continued popularity and wholesomeness of this specialty ethnic fermented meat and will inform and engage the next generation of "salumists" within and beyond NEPA.

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