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Viability of *Escherichia coli* O157:H7 in Fermented Semidry Low-Temperature-Cooked Beef Summer Sausage

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ABSTRACT

The population of inoculated *Escherichia coli* O157:H7 was monitored during the manufacture and storage of a semidry beef summer sausage processed by fermentation and cooking at a low temperature by heating to an internal temperature of 130°F (54°C). The all-beef batter (11% fat and nonmeat ingredients) was inoculated with the commercial starter culture *Pediococcus acidilactici* HP (≥8.6 log CFU/g of batter) and a five-strain mixture of *E. coli* O157:H7 (≥7 log CFU/g) and then hand stuffed into 2.5-inch (64-mm) diameter fibrous casings. The sausages were fermented at an initial temperature of 85°F (29°C) to a final temperature of 105°F (41°C) over ca. 13 h at 80% relative humidity (RH) to pH 4.6 or pH 5.0. After fermentation to pH 4.6, the internal temperature of the chubs was raised to 130°F (54°C) instantaneous over 3.6 h at 60% RH. After fermentation to pH 5.0, the internal temperature of the chubs was raised to 130°F (54°C) over 3.6 h at 60% RH and the chubs were maintained under these conditions for 0, 30, or 60 min. The chubs were cold water showered for 15 min and then chilled at 39°F (4°C) for 6 h before being vacuum packaged and stored at 39°F (4°C) or 77°F (25°C) for 7 days. Regardless of the target pH, fermentation alone resulted in only a 1.39-log CFU/g decrease in pathogen numbers. However, fermentation to pH 4.6 and heating to an internal temperature of 130°F (54°C) instantaneous reduced counts of *E. coli* O157:H7 by ≥7.0 log units to below detection levels (<10 CFU/g). Pathogen numbers remained below levels detectable by direct plating, but viable *E. coli* O157:H7 cells were recovered by enrichment of samples during sausage storage at either refrigeration or abuse temperatures. In contrast, fermentation to pH 5.0 and heating to an internal temperature of 130°F (54°C) instantaneous resulted in a 3.2-log-unit decrease in counts of *E. coli* O157:H7. No appreciable reductions in pathogen numbers were observed thereafter following storage at either 39°F (4°C) or 77°F (25°C) for 7 days. Fermentation to pH 5.0 and heating to an internal temperature of 130°F (54°C) instantaneous followed by holding for 30 or 60 min resulted in about a 5- or 7-log reduction, respectively, in pathogen numbers. For chubs held for 30 min at 130°F (54°C), pathogen numbers decreased to 2.02 and <1.0 log CFU/g at 39°F (4°C) and 77°F (25°C), respectively, after 7 days; viable cells were only observed by enrichment after storage at 77°F (25°C). For chubs held for 60 min at 130°F (54°C), pathogen numbers remained below levels detectable by direct plating, but viable cells were recoverable by enrichment after 7 days at both storage temperatures. These data will be useful guidelines to manufacturers for developing processing conditions to further ensure the safety of this category of fermented sausages relative to food-borne pathogens such as serotype O157:H7 strains of *E. coli*.

Key words: *Escherichia coli* O157:H7, beef, summer sausage, pathogen, fermentation

Escherichia coli O157:H7 has received considerable attention in the United States in recent years as the causative agent of bacterial food-borne diarrheal illness which has an estimated $216 to 580 million in attendant annual costs *(16)*. The 1994 outbreak in Washington involving 20 individuals, including a 6-year-old with hemolytic uremic syndrome (HUS), and 3 individuals in California, including a 4-year-old with HUS, due to consumption of dry-cured salami contaminated with *E. coli* O157:H7 *(7)*, was largely responsible for the United States Department of Agriculture Food Safety and Inspection Service mandate for manufacturers of dry and semidry fermented sausage to ensure a 5-log reduction in numbers of this pathogen during processing *(20)*. As a result of the salami outbreak, the 1993 multistate outbreak due to contaminated hamburger patties with >500 confirmed cases and four deaths *(6)*, and several other illnesses in the 1990s involving foods *(14)*, the following programs and regulations were implemented in the United States to ensure the safety of consumers with respect to food-borne illness due to enterohemorrhagic strains of *E. coli*. The temperature for cooking hamburger patties was raised from 140°F (60°C) to 155°F (68°C) for 16 s *(10)*; safe-handling labels were required for all raw meat and poultry, as well as for partially cooked meats such as bacon *(11)*; a pathogen reduction program was initiated for the meat and poultry industries *(12)*; *E. coli* O157:H7 was declared to be an adulterant when present in ground beef *(1)*; and a ground beef sampling program was instituted at federally inspected processing plants and retail establishments *(2)*.

With the exception of recent studies involving pepperoni *(15)* and salami *(9, 13, 18)*, relatively little information has been published on the validation of processes for control of *E. coli* O157:H7 directly in fermented meats. These studies of pepperoni and salami confirmed that

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fermentation and/or drying and/or storage were only sufficient to deliver a 1- to 2-log reduction in pathogen numbers. Additional steps, such as postfermentation heating and/or extended storage at low pH and/or elevated temperatures, were required to achieve the mandated 5-log reduction of the pathogen *(15, 18)*. To provide additional information on the fate of *E. coli* O157:H7 in fermented meat, in this study the viability of the pathogen was monitored in low-temperature-cooked summer sausage. This category of fermented meat is typically prepared by using beef with fat at levels of ≤30%. However, sometimes it is prepared by using beef in combination with lesser quantities of pork, poultry, venison, and/or bison. Traditionally, summer sausage is manufactured by fermenting the batter for 8 to 24 h at 80 to 110°F (27 to 43°C) and then heating it to 120 to 125°F (49 to 52°C) instantaneous *(8)*. If the batter contains pork, then the raw pork must first be certified by freezing, e.g., at 5°F (−15°C) for 20 days or the resulting chubs must receive a postfermentation heating to an internal temperature of 144°F (62°C) instantaneous *(3)*. After fermentation, the summer sausage is classified as either "uncooked," with internal chub temperature of ≤135°F (57°C) instantaneous, or "cooked," with internal chub temperature of >135°F (57°C) instantaneous, depending on the postfermentation heating regimen that is applied *(24)*. The cooked types greatly predominate, as 90% of the total semidry fermented meat produced in the United States, in commercial production, with uncooked summer sausages produced primarily by smaller scale processors (R. Rust, personal communication). The potential lack of adequate postfermentation heating for uncooked, or perhaps more appropriately named low-temperature-cooked summer sausage to eliminate some serotype O157:H7 strains of *E. coli*, the sizeable volume of this category of product produced (e.g., 10 to 20 million pounds in 1992; L. Duewer, personal communication), and a 1995 outbreak in South Australia (effecting 23 individuals, all with HUS) *(8)* due to a low-temperature-cooked fermented sausage contaminated with a serotype O111:NM verotoxigenic strain of *E. coli*, collectively prompted us to investigate the fate of enterohemorrhagic strains of *E. coli* O157:H7 in low-temperature-cooked summer sausage.

MATERIALS AND METHODS

An overview of the manufacturing process used in this study to prepare fermented semidry low-temperature-cooked beef summer sausage is provided in Figure 1. The present study was conducted using the methods previously described *(15)* with the following notable exceptions. Strain EC505B (beef isolate, Food Research Institute culture collection) was used in the five-strain cocktail of *E. coli* O157:H7 instead of strain SLH21788 (human isolate from Wisconsin day care center outbreak of 1994 *(19)*; and the meat block was all beef (11% fat) and contained the following nonmeat ingredients per 50 lb (22.7 kg): 567.5 g (2.5%) of salt; 227 g (1%) or 90 g (0.3%) of dextrose, to achieve a target pH of 4.6 or 5.0, respectively; 60 g (0.26%) of curing salt (6.25% sodium nitrite) (F. W. Witt & Co, Yorkville, IL); 135 g (0.59%) of commercial summer sausage seasoning mixture (F. W. Witt); and 12.3 g (0.054%) of sodium erythorbate (F. W. Witt).

The meat block was inoculated with the five-strain cocktail of

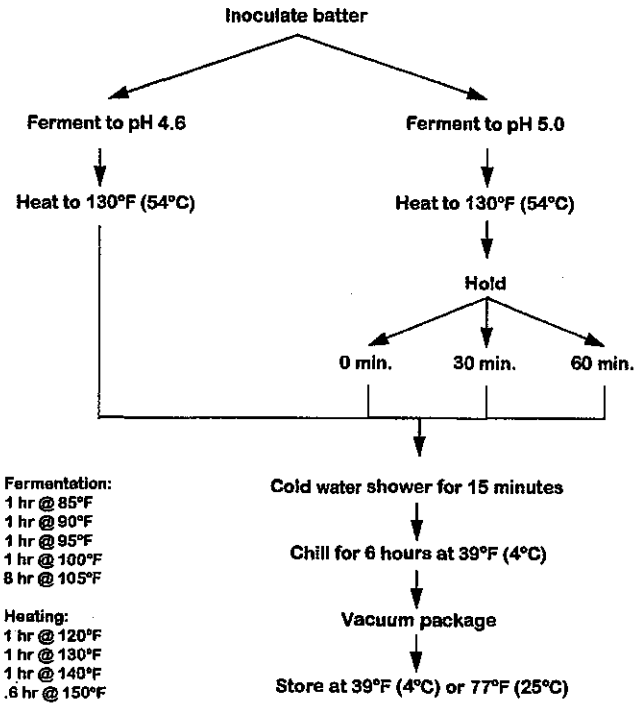

FIGURE 1. *Manufacture of fermented semidry all-beef low-temperature-cooked summer sausage. The temperature regimens listed in the sidebar for fermentation and heating are smokehouse temperatures.*

E. coli O157:H7 to a final concentration of 7.88 ± 0.43 log CFU/g of batter and mixed in a Buffalo paddle mixer (model 2VSS, John E. Smith's and Sons Co., Buffalo, NY) for 1 min. Eleven milliliters of thawed pediococcal starter, strain HP (Diversitech, Inc., McFarland, WI) diluted in 89 ml of sterile distilled H_2O were added to deliver about 8 log CFU/g of batter. Finally, the nonmeat ingredients were added to the batter and mixing was continued for 2 min more. The inoculated batter was removed from the mixer and ground through the 1/8-in. (ca. 0.32 cm) die plate of a Hobart grinder (Model 84142; Hobart Manufacturing Co., Troy, OH). Ground batter was stuffed into 2.5 in. (64 mm) diameter fibrous casings (Vista International Packaging Inc., Kenosha, WI) by using a hand stuffer (Koch Supplies, Inc., Kansas City, MO) and sealed with a casing clipper (Poly-Clip model SCH 7210; Niedecker, West Germany) loaded with series 700-VSCS staples (U.S. Clip Corporation, Mundelein, IL).

Fermentation was initiated at 85°F (29°C) with a relative humidity (RH) of 80%. Over the first 4 h, the temperature of the smokehouse (model 1000; Vortron, Inc., Beloit, WI) was raised to 105°F (41°C) by increasing the temperature 5°F/h while keeping the RH constant, and the fermentation was then continued for another 8 h until pH 4.6 or 5.0 was achieved. Because the smokehouse was substantially underloaded with the sausage product, the fermentation temperature was raised gradually to more closely simulate sausage temperature changes which would occur in a fully loaded industrial smokehouse. The chubs were not actually smoked due to the limitations of our smokehouse. Although it is likely that smoke might have an appreciable effect on the pathogen on the surface of chubs, it is unlikely to appreciably affect cells within chubs *(15)*. Smoking alone would not be sufficient to effect a 5-log reduction in pathogen numbers. Following fermentation, to achieve an internal chub temperature of 130°F (54°C) the dry bulb temperature of the smokehouse was maintained 1 h at 120°F (49°C), 1 h at 130°F (54°C), 1 h at 140°F (60°C), and

35 min at 150°F (66°C), all at 60% RH. After the internal chub temperature of 130°F (54°C) was attained, the chubs were held at this temperature for 0, 30, or 60 min and then cold water showered for 15 min. After showering, the internal temperatures of the chubs decreased to 93 to 108°F (34 to 42°C) depending on the holding time at 130°F (54°C) in the smokehouse. The chubs were then chilled for 6 h at 39°F (4°C) before being vacuum packaged in oxygen-impermeable bags (863 saran, Curwood, Inc., New London, WI) using a Multivac type AGW (Koch Inc., Kansas City, Missouri) apparatus. Finally, vacuum-packaged chubs were stored at 39°F (4°C) or 77°F (25°C) for 7 days. Both experiments (fermentation to pH 4.6 and to pH 5.0) were conducted on 3 different days using 3 different batches of raw meat (3 trials for each experiment). Samples (25 g) were taken from each of 3 chubs at each sampling interval: before stuffing, after fermentation, after cooking, and after storage and plated onto 2 plates of MacConkey sorbitol agar (MSA) (Difco Laboratories Inc., Detroit, MI) from each of 2 tubes of a given dilution essentially as described *(15)*. For samples containing the pathogens at levels $<10^1$ CFU/g of batter, the presence or absence of the pathogen was determined by enrichment as described *(15)*.

Chemical analyses were performed by Silliker Laboratories (Madison, WI) by using AOAC-approved methods *(17)* for pH, titratable acidity, salt, protein, fat, moisture, and a_w on 2 chubs from each trial after storage for 7 days at 39°F (4°C). Statistical analyses were performed by using Statistical Analysis System software (SAS Institute, Cary, NC) to determine the means of the viable counts of *E. coli* O157:H7 and of the chemical composition parameters of the meat.

RESULTS

Microbiological analyses of the raw meat

None of the 6 meat blocks used in this study to prepare batter for summer sausage contained *E. coli* O157:H7 (<10 CFU/g of batter) by direct plating. The mean total aerobic counts and lactic acid bacteria counts of these 6 meat blocks were 1.1×10^4 CFU/g (range, 3.8×10^3 to 2.7×10^4 CFU/g) and 1.5×10^3 CFU/g (range, 4.1×10^2 to 6.8×10^3 CFU/g), respectively. These data indicate meat of good microbiological quality.

Fermentation at 105°F (41°C) to pH 4.6 and heating

Fermentation to pH 4.6 yielded a 1.39-log-unit reduction of the number of *E. coli* O157:H7 CFU in chubs of summer sausage (Table 1). Subsequent heating to an internal temperature of 130°F (54°C) instantaneous reduced pathogen numbers to nondetectable levels (<10 CFU/g of batter) as determined by direct plating, but viable cells of *E. coli* O157:H7 were still recovered by enrichment. Cells of this pathogen were also recovered by enrichment after 1 week of storage at 39°F (4°C) or 77°F (25°C). These data revealed that fermentation to pH 4.6 was not sufficient to deliver a 5-*D* reduction of the pathogen. However, postfermentation heating to an internal temperature of 130°F (54°C) provided a ≥7-log-unit reduction of the pathogen (Table 1), without causing any discernible changes in the appearance or composition of the chubs heated to 130°F (54°C) and held for 0 min (Table 2).

Fermentation at 105°F (41°C) to pH 5.0, heating, and holding

Results from a preliminary trial (data not shown) suggested that *E. coli* O157:H7 in summer sausage fermented to pH 5.0 would not be sufficiently destroyed (i.e., a 5-*D* reduction) by postfermentation heating to an internal temperature of 130°F (54°C) instantaneous. Therefore, after fermentation to pH 5.0, we evaluated the effect of heating the chubs to 130°F (54°C) and then holding the chubs at this temperature for 30 or 60 min on the viability of the serotype O157:H7 cocktail.

Fermentation to pH 5.0 delivered an 0.31-log-unit reduction of the pathogen, and subsequent heating to an internal temperature of 130°F (54°C) instantaneous decreased the number of *E. coli* O157:H7 CFU an additional 2.89 log units to 4.58 log CFU/g (Table 1). After 1 week of storage at either 39°F (4°C) or 77°F (25°C), no extensive further reduction of the pathogen was observed. However, if after fermentation to pH 5.0 the chubs were heated to an internal temperature of 130°F (54°C) and then held at this temperature for 30 min, levels of *E. coli* O157:H7 decreased from an initial level of 7.78 to 2.78 log cfu/g. After 1 week of storage at 77°F (25°C), counts of *E. coli* O157:H7 decreased to below detection (<10 CFU/g) by direct plating, but viable cells were recovered by enrichment. Counts of the pathogen remained relatively constant in chubs stored at 39°F (4°C). Similarly, holding the chubs at an internal temperature of 130°F (54°C) for 60 min following fermentation to pH 5.0 yielded a ≥7.0-log-unit reduction of the pathogen, but again, viable cells of the pathogen were detectable following enrichment. After 7 days of storage at either 39°F (4°C) or

TABLE 1. *Population of inoculated* E. coli *O157:H7 in fermented semidry low-temperature-cooked summer sausage during manufacture*

	log *E. coli* O157:H7 CFU/g of sausage (mean ± SD; $n = 3$) at manufacturing step:			
			After storage for 7 days at:	
Batter[a]	After fermentation to (pH)	After heating to 130°F (54°C) and holding for (min)	39°F (4°C)	77°F (25°C)
7.99 ± 0.33	6.60 ± 0.44 (pH 4.6)	(0) <1.0[b]	<1.0[b]	<1.0[b]
7.78 ± 0.43	7.47 ± 0.26 (pH 5.0)	(0) 4.58 ± 0.46	4.74 ± 0.54	4.14 ± 0.65
		(30) 2.78 ± 0.40	2.02 ± 0.83	<1.0[b]
		(60) <1.0[b]	<1.0[b]	<1.0[b]

[a] The pH of the raw batter was ca. pH 5.84 and ranged from 5.95 to 5.77 over the three trials.
[b] Not detectable by direct plating methods (<10 cfu/g) but detectable by enrichment.

TABLE 2. *Comparison of the physicochemical parameters of differently processed semidry low-temperature-cooked summer sausage after 7 days of storage at 4°C*

	Sausage physicochemical parameters[a] (mean ± SD, $n = 3$)							
Proce	pH	TA (%)	M/Pr	a_w	Salt (%)	Moisture (%)	Protein (%)	Fat (%)
Experiment I[b]	4.53 ± .04	1.90 ± .02	3.16 ± .08	0.94 ± .00	2.45 ± .12	62.98 ± 2.12	19.66 ± .36	11.95 ± 1.08
Experiment II[c]	4.90 ± .09	1.55 ± .18	3.06 ± .04	0.95 ± .00	2.61 ± .05	64.87 ± .83	20.98 ± .19	10.06 ± .35

[a]

[b] Fermentation to pH 4.6 plus heating to 130°F internal temperature instantaneous.

[c] Fermentation to pH 5.0 plus heating to 130°F internal temperature and holding for 60 min.

77°F (25°C), it was only possible to recover the pathogen by enrichment. These data revealed that a 5-*D* reduction was only achieved if chubs fermented to pH 5.0 were heated to an internal temperature of 130°F (54°C) and then held for 30 (ca. 5.0-log reduction) or 60 (ca. 7-log reduction) min. As shown in Table 2, heating did not appreciably alter the compositional attributes of the chubs heated to 130°F (54°C) and held for 60 min.

DISCUSSION

Reports indicate that *E. coli* O157:H7 ranks third behind *Salmonella* and *Campylobacter* species as a leading cause of bacterial food-borne diarrheal cases worldwide *(23)* and ranks fourth among the most costly food-borne diseases in the United States *(16)*. From a food safety standpoint, this pathogen is of great concern due to the low infectious dose, the potential life-threatening complications of the ensuing disease, and the ability to tolerate relatively low pH (high acid) conditions. There is also concern about potential ambiguities encountered with detection of *E. coli* O157:H7 relative to other coliforms due to its poor growth at ≥42°C, inability to ferment sorbitol, and lack of β-glucuronidase activity *(14)*. The ability to withstand low pH conditions may explain, at least in part, the survival of this pathogen in certain fermented meat products. Prior to the Washington and California salami outbreak of late 1994, fermented meats were generally regarded as safe due to the presence of organic acid(s) and a low pH, as well as the cooking, curing, smoking, drying, vacuum packaging, and/or refrigerated storage such products may receive. Published studies revealed that fermented meats can serve as a vehicle of transmission for O157:H7 strains *(22)* and that serotype O157:H7 strains of *E. coli* were not appreciably affected (i.e., ≤2-log decrease) by standard fermentation and drying regimens *(15, 18)*. Additional processing steps, such as postfermentation heating, were required to achieve an appreciable reduction in pathogen numbers. For example, Hinkens et al. *(15)* reported that heating pepperoni chubs to an instantaneous internal temperature of 145°F (63°C) or 128°F (53°C) for 60 min was sufficient to effect a 5-*D* kill of an *E. coli* O157:H7 cocktail without visibly affecting the texture or appearance of the chubs. However, not all summer sausage, the category of fermented meat evaluated in the present study, is heated to an internal temperature of 145°F (63°C) or 128°F (53°C) for 60 min due to perceived alterations in the texture and/or flavor of the resulting product (R. Rust, personal communication). More specifically, 22 processors participating in the 1996 product show sponsored by the Wisconsin Association of Meat Processors (WAMP) each entered at least one summer sausage product into the low-temperature-cooked class. This category is delineated by postfermentation heating at internal chub temperatures of ≤135°F (57°C) *(24)*. An informal survey of contributing processors at the WAMP Product Show revealed that most low-temperature-cooked summer sausage chubs were heated to temperatures ranging from 128°F (53°C) to 135°F (57°C). The paucity of information on the fate of enterohemorrhagic *E. coli* in summer sausage in general, the outbreak in Australia due to "uncooked" summer sausage *(8)*, and the identification of summer sausage as a product with a high priority for validation using USDA/FSIS criteria, e.g., high pH, high moisture/protein ratio, and all beef ingredients *(21)* were the impetus for the present study to evaluate pathogen viability during manufacture and storage of low-temperature-cooked ("uncooked") beef summer sausage.

Several factors, e.g., fermentation temperature and time, casing size, postfermentation heating, pH, and type of organic acid may influence the viability of cells of *E. coli* O157:H7 in fermented sausage. As discussed above, postfermentation heating resulted in a ≥5-log reduction of pathogen numbers *(15)*. As another example, Nickelson et al. *(18)* demonstrated that the pathogen was reduced to a greater degree in smaller diameter (55 mm; 6.43-log CFU/g decrease) than larger diameter (105 mm; 4.72-log cfu/g decrease) casings when salami was fermented at 90°F (32°C) to pH 4.6 and held at 90°F (32°C) for 6 to 7 days. It is possible that pathogen numbers were reduced to a greater extent in smaller casing chubs due to a higher rate of heat penetration with higher fermentation temperatures in combination with high-acid conditions possibly sensitizing cells of *E. coli* O157:H7 to subsequent stress during manufacture or storage *(15, 18)*. For example, higher [110°F (43°C)] fermentation temperatures typically provided a greater reduction in pathogen numbers than lower [70°F (21°C)] fermentation temperatures for similar manufacturing processes *(18)*. Likewise, extended holding of chubs after fermentation at an elevated temperature was more effective at reducing pathogen numbers than an otherwise similar fermentation at a lower pH *(18)*. In addition to pH, the antimicrobial effects of organic acids may affect the survival of the pathogen in fermented meats, especially at pH levels near the pK_a of lactic acid. The intrinsic acid tolerance of the pathogen may

also have an impact on survival characteristics; therefore, stationary-phase cells of serotype O157:H7 may survive in greater numbers in fermented meats than log-phase cells *(4)*.

In the present study, we evaluated the viability of a cocktail of serotype O157:H7 strains of *E. coli* in summer sausage manufactured by using conditions that mimic what is practiced by the sausage-processing industry. Fermentation at 105°F (41°C) to pH 4.6 or 5.0 and postfermentation heating of chubs to an internal temperature of 130°F (54°C) for 30 min delivered a >5-log decrease in pathogen numbers. Additional studies are warranted to better quantify the effects of postfermentation heating and holding on product quality, appearance, and composition and to evaluate additional formulations and/or intervention strategies to eliminate this pathogen. In addition to a hazard analysis critical control points program and good manufacturing practices, the data herein provide manufacturers of fermented, semidry, low-temperature-cooked beef summer sausage with time and temperature guidelines for ensuring safety relative to *E. coli* O157:H7.

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