Journal of Food Protection 86 (2023) 100088

Contents lists available at ScienceDirect

Journal of Food Protection

journal homepage: www.elsevier.com/locate/jfp

Research Paper

Fate of Shiga Toxin-Producing *Escherichia coli* (STEC) and *Salmonella* during Kosher Processing of Fresh Beef

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ARTICLE INFO

Keywords:
Fresh beef
Kosher processing
Salmonella
STEC

ABSTRACT

Traditional kosher meat processing involves the following steps after slaughtering: soaking with water to remove blood, salting to help draw out more blood, and rinsing to remove salt. However, the impact of the salt used on foodborne pathogens and beef quality is not well understood. The objectives of the current study were to determine the effectiveness of salt in reducing pathogens in a pure culture model, on surfaces of inoculated fresh beef during kosher processing, and the effect of salt on beef quality. The pure culture studies indicated that the reduction of *E. coli* O157:H7, non-O157 STEC, and *Salmonella* increased with increasing salt concentrations. With salt concentrations from 3 to 13%, salt reduced *E. coli* O157:H7, non-O157 STEC, and *Salmonella* ranging from 0.49 to 1.61 log CFU/mL. For kosher processing, the water-soaking step did not reduce pathogenic and other bacteria on the surface of fresh beef. Salting and rinsing steps reduced non-O157 STEC, *E. coli* O157:H7, and *Salmonella* ranging from 0.83 to 1.42 log CFU/cm^2, and reduced Enterobacteriaceae, coliforms, and aerobic bacteria by 1.04, 0.95, and 0.70 log CFU/cm^2, respectively. The salting process for kosher beef resulted in reducing pathogens on the surface of fresh beef, color changes, increased salt residues, and increased lipid oxidation on the final products.

Kosher slaughter of animals and meat processing is carried out in accordance with rabbinic law, in which stunning before slaughter is not acceptable (Anil, 2012). The animal must be conscious at the time of slaughter and may only be performed by a certified person called a "shochet". After the slaughtering, the animals are gutted and skinned. At this point, certain major veins and arteries are removed from meat animals such as cows and sheep, a process known as "nikkur". According to Jewish dietary laws (Angel, 1994), koshering is the final step in the process of making meat fit for the consumption by Jewish consumers. In koshering, blood is removed from the meat, and then the meat is soaked in water for at least half an hour, salted with coarse salt known as "melichah" or salting for 45–60 min, and finally by triple rinsing (Regenstein & Regenstein, 1988), which has been practiced for over 3000 years and for the most part has not changed. It is widely known that beef carcasses can become contaminated with pathogens and spoilage bacteria during the harvesting process, particularly up to and during hide removal (Bell, 1997; Brichta-Harhay et al., 2008). Cross-contamination, posthide removal as well as during fabrication, can also occur (Villarreal-Silva et al., 2016). Keeping products safe and free from contamination with pathogenic bacteria such as Shiga toxin-producing *Escherichia coli* (STEC) and *Salmonella* is one of the greatest challenges for the meat industry. The Centers for Disease Control and Prevention (CDC) estimate the approximate annual number of illnesses due to *Salmonella*, *Escherichia coli* O157:H7, and six serogroups non-O157 STEC as 1.2 million, 97,000, and 170,00 illnesses, respectively (Scallan et al., 2011).

Numerous thermal and chemical interventions to reduce at least 90% of STEC and *Salmonella* have been successfully developed and implemented to reduce the risk of contamination of meat and meat products during the conventional harvesting process (Koohmaraie et al., 2007; Wheeler et al., 2014). However, potential microbial contamination by foodborne pathogens and the efficacy of pathogen reduction during kosher meat processing have not been systematically studied. Steam and hot water cannot be applied to reduce microbial contamination until after the meat has been salted due to heat denaturing of meat, trapping the blood within the flesh (Marsden, 2013; Rastogi, 2010). The Egyptians were the first to realize the preservation possibilities of salt (Butler, 2013). Salt draws the moisture out of foods, drying them and potentially making it possible to store meat without refrigeration for extended periods of time. However, information on

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https://doi.org/10.1016/j.jfp.2023.100088
Received 23 September 2022; Accepted 29 March 2023
Available online 3 April 2023
0362-028X/Published by Elsevier Inc. on behalf of International Association for Food Protection.

the impact of the kosher beef salting process on foodborne pathogens and beef quality is very limited.

There also are significant health problems associated with the consumption of excess salt. Excess sodium increases the risk of high blood pressure, stroke, and cardiovascular disease, the leading cause of death in the United States (CDC, 2017). The recommended upper limit of sodium intake for adults is 2,300 mg per day, and the estimated average intake for adult females is over 3,000 mg per day and about 4,500 mg per day for adult males (HHS & USDA, 2015).

Commercial safety data regarding the kosher beef market is very limited because the United States Department of Agriculture (USDA) does not keep statistics on kosher slaughter. Moreover, most of the kosher packers, processors, and distributors are privately held companies, which do not publish their business information in the public domain. Many antimicrobial interventions cannot be applied due to kosher restrictions. Therefore, the objectives of this study were to determine the effectiveness of salt on the kosher process in reducing pathogens on surfaces of fresh beef, and the effect of added salt on beef quality.

Materials and methods

Bacterial strains, growth conditions, and preparation of inocula. Two isolates each of non-O157 STEC serotypes O26:H11, strains 3392 and 3891; O45:H2 strains 01E-1269 and O45 WDG3; O103:H2, strains 2421 and G5550637; O111:NM, strains 1665 and 3007:85, O121:H7 and O121:H19 02E-2074; O145:NM and O145 GB; O157:H7, three isolates of *Escherichia coli* O157:H7, strain ATCC 43895, FSIS 3, and FSIS 4; two isolates each of *Salmonella* Newport, strains 13109 and 15124; Typhimurium, strains 14218 and DT-104 from the United States Meat Animal Research Center (USMARC) culture collection were grown individually and statically for 16 to 18 h at 37°C in nutrient broth (Becton and Dickinson, Detroit, MI). All non-O157 STEC strains were isolated from human cases except strains O45 WDG3, O121:H7, and O145 GB which were isolated from bovine animals. Three *E. coli* O157:H7 strains were isolated from hemorrhagic colitis outbreaks. All *Salmonella* strains were isolated from bovine animals. Each strain was adjusted with nutrient broth (BD) to a cell concentration of approximately 1.5×10^8 CFU/mL using a precalibrated spectrophotometer with McFarland equivalence turbidity standards (ThermoFisher Scientific) at 600 nm with the absorbency between 0.08 and 0.1. Three inocula were formulated for the pure culture study by mixing an equal volume of each strain. For the fresh beef inoculation study, these inocula were 10-fold diluted with maximum recovery diluent (MRD; Difco, BD). Inoculum I was composed of all twelve non-O157 STEC serotypes O26, O45, O103, O111, O121, and O145. Inoculum II was composed of *E. coli* O157:H7 strains ATCC 43895, FSIS 3, and FSIS 4, while the inoculum III was composed of two strains of *S.* Newport and two strains of *S.* Typhimurium (Table 1). All inocula were prepared within 30 min and kept in an ice bath to prevent cell proliferation for 30 min before use in the studies.

Pure culture study for different kosher salt concentrations treatment. A stock salt solution (30% w/v) was prepared by weighing 30 g of kosher salt (Cargill Incorporated) and brought the volume to 100 mL. The well-mixed salt solution was autoclaved for sterilization and cooled to room temperature before use. To determine the effectiveness of salt on reducing the target pathogens, each inoculum was subjected to different salt concentrations of 0, 3, 6, and 13% (Table 2) using a 2-mL cluster tube (VWR) and incubated at 4°C for 1 h before enumeration. The temperature of 4°C was selected based on slowing the growth of bacterial cells in untreated control samples. Two tubes were used for each inoculum, and three repetitions were conducted for this study. Each bacterial salt solution was serially diluted 10-fold with maximum recovery diluent (MRD; Difco, BD) and surface plated on selective agar media USMARC chromogenic agar (UCA; Kalchayanand et al., 2013) and xylose lysine deoxycholate (XLD; Remel) and nonselective medium tryptic soy agar (TSA; Difco, BD) using a spiral plater (Spiral Biotech). All plates were incubated at 37°C for 24–36 h for enumeration. A nonselective medium was used to enumerate the injured bacterial cells inflicted from the salt treatment that may have not survived on the selective medium. Presumptive colonies of STEC and *Salmonella* were confirmed using both agglutination tests (Oxoid, Remel) and multiplex PCR (Kalchayanand et al., 2013; Paton & Paton, 1998; Perelle et al., 2004; Rahn et al., 1992).

Fresh beef preparations and inoculation. Fifteen fresh beef flanks (cutaneous trunci muscle) were collected from a local beef cattle

Table 2
Different kosher salt concentrations treatment set up in pure culture study

Target conc.	Stock NaCl (30%)	Sterile water	2X nutrient broth	Inoculum
0%	0 µL	450 µL	450 µL	100 µL
3%	108.25 µL	341.75 µL	450 µL	100 µL
6%	216.5 µL	233.5 µL	450 µL	100 µL
13%	433 µL	17 µL	450 µL	100 µL

Table 1
Bacterial strains and inoculum information

Bacterial species	Serotype	Strain/source[a]	Colony color on USMARC Chromogenic agar	Inoculum group
Escherichia coli	O26:H11	3392/Hum.	Turquoise	1
Escherichia coli	O26:H11	3891/Hum.	Turquoise	1
Escherichia coli	O45:H2	01E-1269/Hum.	Light blue green	1
Escherichia coli	O45	WDG3/Bov.	Light blue green	1
Escherichia coli	O103:H2	2421/Hum.	Light green	1
Escherichia coli	O103:H2	G5550637/Hum.	Light green	1
Escherichia coli	O111:NM	1665/Hum.	Dark blue green	1
Escherichia coli	O111:NM	ECRC3007/Hum.	Dark blue green	1
Escherichia coli	O121:H7	GB/Bov.	Small purple	1
Escherichia coli	O121:H19	02E-2074/Hum.	Light blue gray	1
Escherichia coli	O145:NM	GS5578620/Hum.	Purple	1
Escherichia coli	O145	GB/Bov.	Purple	1
Escherichia coli	O157:H7	ATCC 43895/Hum.	Hunter green	2
Escherichia coli	O157:H7	FSIS 3/Hum.	Hunter green	2
Escherichia coli	O157:H7	FSIS 4/Hum.	Hunter green	2
Salmonella	N/A	Newport 13109/Bov.	Colorless	3
Salmonella	N/A	Newport 15124/Bov.	Colorless	3
Salmonella	N/A	Typhimurium 14218/Bov.	Colorless	3
Salmonella	N/A	Typhimurium DT-104/Bov.	Colorless	3

[a] Source; Hum., human isolate; Bov., bovine isolate. N/A, not applicable.

processing plant, vacuum-packaged, and stored at −20°C until use. For each repetition, five frozen beef flanks were thawed at 2–4°C and each flank with approximately 2.5 cm thickness was aseptically cut into 5 by 10 cm (approximate 100 g) pieces for a total of 20 pieces. The 20 pieces of fresh beef were equally divided into five groups as follows: (1) untreated control, (2) water soaked, (3) water soak and chilled, (4) salted and rinsed, and (5) salted, rinse, and chilled. Three repetitions were conducted for the study. Each inoculum was applied individually before subjecting it to kosher processing on one side each of the 20 fresh beef tissues, and the inoculum was spread over the entire area using a sterile disposable hockey stick. The concentration of the target bacteria was approximately 10^4–10^5 CFU/cm^2. The inoculated fresh beef tissues were held at 4°C for 15–20 min for bacterial cell attachment before kosher processing. The holding time of 15–20 min was based on evidence that most attachment of *E. coli* occurs between 1 and 20 min over a wide temperature range (2.5–37°C) with little increase occurring after 20 min (Butler et al., 1979).

Kosher processing and sample collection. Before kosher processing, four inoculated fresh beef tissues were placed individually in filter bags as untreated control samples and enumerated for an initial population. In this study, the kosher processing steps followed one of the commercially produced kosher beef plants in the United States with slight modifications to accommodate handling in the laboratory. Sixteen pieces (50 cm^2 each) of inoculated fresh beef were soaked in tap water (four pieces per 1 L) for 45 min. After soaking, the excess liquid was allowed to drip off the beef tissues for 30 s on a stainless steel rack. Four pieces of beef tissue were individually placed in sterile filter bags for enumeration to determine the populations after water soaking. Another four pieces of beef tissues were placed each piece individually in filter bags and stored at 4°C for 48 h before enumeration to determine the population after water soaking and chilling. For the other eight pieces, each piece (50 cm^2) of water-soaked and dripped off the excess liquid of beef tissues was transferred to a weigh boat containing 75 g of kosher salt. Each piece of beef was rolled until entirely covered with kosher salt with slight rubbing on all exposed meat surfaces and incubated for 45 min at 4°C. The amount of salt required to cover all the exposed meat tissue was predetermined to be 75 g/piece for consistency throughout the study. After the salting of eight beef tissues was completed, each piece of the tissue was randomly held with tongs and shaken for 5 s under 2 L of tap water in a stainless steel tub to remove most of the salt. The eight pieces of washed beef tissues were placed and held in the stainless steel tub for 2 min for the first rinse. After 2 min, each piece of beef tissue was then randomly shaken under the water of the same tub for five s and placed in another stainless steel tub containing 2 L of tap water for the second rinse. The third rinse was repeated after the second rinse, and the excess liquid from the eight beef tissues was allowed to drip off for 30 s on a stainless steel rack before placing each piece, individually, in filter bags. Four filter bags containing beef tissues were enumerated immediately, while the other four bags were stored aerobically at 4°C for 48 h before enumeration, to determine the survivors after salting, rinsing, and chilling. Three repetitions were conducted for the study.

Enumeration and culturing. A 150-mL aliquot of buffered peptone water (Difco, BD) was added into each filter bag containing beef tissue (50 cm^2) and was homogenized for 2 min using a stomacher (BagMixer® 400; Interscience). Each homogenate was 10-fold serially diluted with MRD. Appropriate dilutions were spiral plated (Spiral Biotech) using UCA for the enumeration of *E. coli* O157:H7 and non-O157 STEC, XLD for the enumeration of *Salmonella*, TSA for the enumeration of aerobic bacteria (AB). For Enterobacteriaceae (EBC) and coliforms (CC), Petrifilm™ for Enterobacteriaceae and coliform count plates (3M Corporation) were used for enumeration, according to the manufacturer's recommendations. The agar plates were incubated at 37°C for 24–36 h before counting *E. coli* O157:H7 and non-O157 STEC (Kalchayanand et al., 2013); *Salmonella*, AB, EBC, and CC. The limit of detection for spiral plate and for Petrifilm™ was 80 and four CFU/cm^2, respectively.

Determination of salt residues and lipid oxidation of kosher beef. To determine the effects of kosher salt on fresh beef quality, 36 fresh beef tissues were divided into two groups, untreated control samples and kosher processed samples. The first group of 18 untreated tissue samples were placed in plastic bags (Whirl-Pak; oxygen transmission rate 450 cc/100 in^2/24 h). Nine sample bags were analyzed immediately for 0 d, and the other nine sample bags were stored for 7 d at 2–4°C. The second group of 18 fresh beef tissues were subjected to kosher processing as described above. The 18 salted-rinsed tissue samples were placed in plastic bags (Whirl-Pak) and stored for 0 and 7 d at 2–4°C. Untreated control and salted-rinsed tissue samples were extracted for the determination of salt residues and lipid oxidation from beef tissues. For salt residues at each time interval, 25 g of untreated control and salted-rinsed beef samples were excised and homogenized with 25 mL of cold phosphate buffer (4°C at pH 6.5) for 2 min using a stomacher. A 5-mL aliquot was filtered through a 0.45-μm membrane filter (Pall Corporation) to eliminate meat and fat particles. The concentration of salt residues of the filtrate was determined using a chloride portable photometer (Hanna Instruments) according to the manufacturer's recommendations. For lipid oxidation, 10-g samples were excised from the untreated control and salted-rinsed beef samples and were homogenized with 10 mL of cold phosphate buffer (pH 6.5) for 2 min at 20,000 rpm using a homogenizer (Tekmar Tissumizer, SDT-181059, Tekmar Co.). The thiobarbituric acid reactive substance (TBARS) analysis was used to measure lipid oxidation from the tissue homogenate according to the manufacturer's recommendations and expressed as milligram of malondialdehyde per kilogram of meat (mgMDA/kg) using a Quantichrom thiobarbituric acid reactive substances (TBARS) assay kit (DTBA-100, BioAssay Systems).

Statistical analysis. The enumeration numbers were log-transformed prior to analysis. Sodium chloride concentration and TABRS lipid oxidation also provided information on whether kosher processing caused changes in organoleptic perceptions related to consumer acceptance. Data, including enumerations, salt residues, and lipid oxidation, were analyzed using one-way analysis of variance (ANOVA) with kosher salt treatment as the main effect. The Tukey-Kramer test was used for the comparison of means between pairs with the probability level at $P \leq 0.05$ using JMP (Statistical Discovery™ from SAS, version 13.1.0).

Results and discussions

Pure culture study. Each inoculum, *E. coli* O157:H7, non-O157 STEC, and *Salmonella*, was treated with different kosher salt concentrations to determine the efficacy of salt in reducing the target microorganisms at different storage times. Reduction of *E. coli* O157:H7, non-O157 STEC, and *Salmonella* increased with increasing concentrations of salt (Table 3). On the nonselective medium, salt concentrations from 3 to 13% significantly reduced *E. coli* O157:H7 from 0.19 to 0.47 log CFU/mL after 1 h exposure at 4°C. Similarly, salt concentrations of 3–13% significantly reduced non-O157 STEC from 0.12 to 0.55 log CFU/mL and reduced *Salmonella* from 0.09 to 0.37 log CFU/mL (Table 3). Madril and Sofos (1985) and Boons et al. (2013) who reported that when levels of salt were increased, bacterial growth decreased.

Enumeration of the target organisms using selective media had similar results as a nonselective medium, but the bacterial reduction from salt treatment was higher (Table 3). On selective media, salt treatment reduced *E. coli* O157:H7, non-O157 STEC, and *Salmonella* from 0.49 to 1.46 log CFU/mL, from 0.50 to 1.61 log CFU/mL, and from 0.58 to 1.39 log CFU/mL, respectively, after 1 h exposure time.

Table 3
Different sodium chloride (NaCl) concentrations against target pathogens

Pathogen[b]	%NaCl (w/v)[c]	n	Population (log CFU/mL)[aa]		
			Non-selective	Selective	log Injury
E. coli O157:H7	0	6	7.11 ± 0.10	6.70 ± 0.12	
	3	6	6.95 ± 0.09 (0.19^{B})[d]	6.21 ± 0.21 (0.49^{C})	0.74 ± 0.18^{C}
	6	6	6.84 ± 0.07 (0.27^{B})	5.81 ± 0.20 (0.89^{B})	1.03 ± 0.23^{B}
	13	6	6.64 ± 0.14 (0.47^{A})	5.23 ± 0.32 (1.47^{A})	1.41 ± 0.24^{A}
nSTEC	0	6	7.03 ± 0.15	6.34 ± 0.65	
	3	6	6.91 ± 0.12 (0.12^{B})	5.83 ± 0.51 (0.51^{C})	1.08 ± 0.50^{B}
	6	6	6.83 ± 0.13 (0.20^{B})	5.30 ± 0.70 (1.04^{B})	1.52 ± 0.75^{AB}
	13	6	6.48 ± 0.38 (0.55^{A})	4.73 ± 0.75 (1.61^{A})	1.76 ± 0.74^{A}
Salmonella	0	6	6.93 ± 0.21	6.00 ± 0.69	
	3	6	6.84 ± 0.23 (0.09^{B})	5.41 ± 0.48 (0.59^{B})	1.43 ± 0.36^{B}
	6	6	6.76 ± 0.25 (0.17^{B})	4.97 ± 0.53 (1.03^{AB})	1.80 ± 0.49^{AB}
	13	6	6.56 ± 0.27 (0.37^{A})	4.60 ± 0.73 (1.40^{A})	1.96 ± 0.61^{A}

ABC Means in the same column within the same type of microorganism at different %NaCl bearing the same letter are not significantly different ($P \geq 0.05$).
[a] Nonselective medium, tryptic soy agar; selective medium, USMARC chromogenic agar medium.
[b] O157, *E. coli* O157:H7; nSTEC, non-O157 Shiga toxin-producing *E. coli*; Sal, *Salmonella*.
[c] NaCl treatment for 1 h at 4°C.
[d] Number in parentheses indicates log reduction.

The pure culture study reductions of *E. coli* O157:H7, non-O157 STEC, and *Salmonella* on the nonselective medium were 0.55 log CFU/mL or less after 1 h with 13% salt. This indicated that salt had low inactivation ability against these pathogens. In contrast, the reductions of these pathogens were between 1.40 and 1.61 log CFU/mL after 1 h with 13% salt when using selective media. This indicated that salt caused an inhibitory effect and inflicted injury to these pathogens (Table 3). Salt treatment for 1 h significantly inflicted injury on *E. coli* O157:H7, non-O157 STEC, and *Salmonella* from 0.74 to 1.41 log CFU/mL, from 1.08 to 1.76 log CFU/mL, and from 1.43 to 1.96 log CFU/mL, respectively.

Kosher processing of fresh beef. For the inoculation study on surfaces of fresh beef, the survivors of non-O157 STEC, *E. coli* O157:H7, and *Salmonella* during the kosher process are presented in Table 4. The initial population of non-O157 STEC, *E. coli* O157:H7, *Salmonella*, Enterobacteriaceae, coliforms, and aerobic bacteria did not change ($P > 0.05$) after soaking for 45 min in tap water, indicating that bacterial cells were firmly attached on surfaces of fresh beef, 20 min after inoculation. However, water soaking for 45 min followed by chilling for 48 h at 4°C significantly reduced the population of non-O157 STEC serogroups O26, O103, O111, O145, *E. coli* O157:H7, *Salmonella*, and Enterobacteriaceae from 0.27 to 0.60 log CFU/cm^2 compared to controls, but elicited no reduction of non-O157 STEC serogroups O45 and O121, coliforms, and aerobic bacteria. When kosher salt was applied for 45 min and then excess salt rinsed off, all non-O157 STEC, *E. coli* O157:H7, *Salmonella*, Enterobacteriaceae, coliforms, and aerobic bacteria populations on surfaces of fresh beef were significantly reduced from 0.70 to 1.42 log CFU/cm^2 compared to control (Table 4). A slight additional reduction of the target organisms was shown after salting, rinsing, and chilling for 48 h at 4°C. Salt treatment reduced serogroups O45, O111, O145, *E. coli* O157:H7, and *Salmonella* slightly more than 1 log CFU/cm^2, while salt treatment reduction was approximately 0.8 log CFU/cm^2 for serogroups O26, O103, and O121. (Hajmeer et al. (2004) inoculated *E. coli* O157:H7 and *Staphylococcus aureus* on surfaces of fresh beef briskets and spray-treated with a 25% salt solution. The salt solution produced a 1 log reduction of *E. coli* O157:H7 but did not reduce *Staphylococcus aureus*. Studies have shown that rubbing salt onto the surface of meat, in the koshering process, reduced *E. coli*, *Salmonella*, coliforms, and aerobic bacteria on beef briskets (Hajmeer et al., 1999), which agreed with the present studies. Salting for 45 min induced stress on the pathogens, leading to some reductions of target microorganisms depending on bacterial strains. Enumeration with non-selective medium both in the pure culture and the inoculation studies also indicated that salt treatment at

Table 4
Bacterial populations on surfaces of fresh beef during kosher processing

Treatment[b]	n	log population (CFU/cm^2)										
		O26	O45	O103	O111	O121	O145	O157	Sal	EBC	CC	AB
Control	12	4.74^{A}	4.64^{A}	4.86^{A}	4.60^{A}	4.76^{A}	4.74^{A}	4.83^{A}	4.58^{A}	5.10^{A}	4.79^{A}	5.32^{A}
Water soaked	12	4.68^{A}	4.64^{A}	4.72^{AB}	4.69^{A}	4.64^{A}	4.51^{AB}	4.76^{A}	4.45^{AB}	4.99^{AB}	4.74^{A}	5.26^{A}
Water soaked-chilled	12	4.47^{B}	4.50^{A}	4.46^{B}	4.10^{B}	4.54^{A}	4.35^{B}	4.23^{B}	4.22^{B}	4.81^{B}	4.58^{A}	5.23^{A}
Salted and rinsed	12	3.88^{C}	3.54^{B}	4.03^{C}	3.57^{C}	3.93^{B}	3.49^{C}	3.41^{C}	3.47^{C}	4.06^{C}	3.84^{B}	4.62^{B}
		(0.86)[c]	(1.10)	(0.83)	(1.06)	(0.83)	(1.25)	(1.42)	(1.11)	(1.04)	(0.95)	(0.70)
Salted rinsed-chilled	12	3.62^{D}	3.51^{B}	3.83^{C}	3.02^{D}	3.74^{B}	3.30^{C}	3.24^{C}	3.20^{C}	3.95^{C}	3.80^{B}	4.44^{C}
		(1.12)	(1.13)	(1.03)	(1.58)	(1.02)	(1.44)	(1.59)	(1.38)	(1.15)	(0.99)	(0.88)

A-D Means in the same column for each bacterial survivor with the same letter are not different significantly ($P > 0.05$).
[a] Serogroups O26, O45, O103, O111, O121, and O145, non-O157 STEC; O157, *E. coli* O157:H7; Sal, *Salmonella*; EBC, Enterobacteriaceae counts; CC, coliform counts; AB, aerobic bacteria.
[b] Control, inoculated without any treatment; water soaked, inoculated, and soaked in water for 45 min; salted and rinsed, covered with kosher salt for 45 min and rinsed three times for 2 min each with water. The chilled beef samples were either soaked or salted and rinsed and stored aerobically at 4°C for 48 h before enumeration. The average background flora of three beef flanks were approximately 2 to 3 × 10^3 CFU/cm^2 using Petrifilm aerobic count plates 3M.
[c] Number in parentheses indicates log reduction.

13% or salted and rinsed reduced target bacteria by less than 1 log (Table 3; 0.37 to 0.55 log CFU/cm^2 and Table 4; 0.70 log CFU/cm^2) compared to water soaking only and could not be considered as an effective killing step.

Salt has a bacteriostatic property and aids in controlling the microbial flora of meat (Desmond, 2006). Salt works as a preservative to reduce or inhibit microbial growth including pathogens, mainly due to associated changes from lower water activity, drawing water from cells of both food and bacteria, and increased ionic strength (Albarracín et al., 2011; Hajmeer et al., 1999). In very high salt solutions, an increase in external cell osmotic pressure causes water efflux and dehydration. When salt is removed, many microorganisms will be ruptured due to the osmotic pressure difference between the outside and inside of the organism. High salt also interferes with a microorganism's enzyme activity and weakens the molecular structure of its DNA (Parish, 2006)

Kosher processing and fresh beef quality. Since salting is a major part of the koshering process, these experiments have evaluated the salt content in meat and the effect of salt on meat quality traits. The effects of kosher processing on salt residues, lipid oxidation, and color of fresh beef are presented in Table 5 and Figure 1. The salt residue was significantly higher from the kosher processed beef samples than from the unsalted control beef samples. There was no difference ($P > 0.05$) between the unsalted control and kosher processed beef samples during storage at 0 and 7 d at 4°C. In the present study, the salt residue from kosher beef was 14 times higher than nonkosher beef (Table 5). Excess sodium intake can be detrimental to human health, including high blood pressure and increased risk of heart attack or stroke (Karppanen & Mervaala, 2006; Roberts, 2001). According to the United States Department of Health and Human Service and United States Department of Agriculture dietary guidelines, the recommended sodium intake for adults is no more than 2,300 mg per day and further reduced to 1,500 mg for people who are 51 and older, African American, or have hypertension, diabetes, or chronic kidney disease (HHS & USDA, 2010). Meat naturally contains less than 100 mg/100 g of sodium, therefore, people 51 and older, those diagnosed with cardiovascular disease, stroke, and coronary heart disease, African Americans, or those who have hypertension, diabetes, or chronic kidney disease must be considered when consuming kosher beef. In most commercial kosher processing facilities, meat is, however, koshered in very large pieces from the forequarter such as shoulder, chuck, rib, and brisket. Since only small pieces (50 cm^2) from cut beef flanks were salted in the present study, the surface area to volume of meat ratio was much larger, which contributed to a larger salt content per 100 g.

The TBARS value, expressed as mg malondialdehyde (MDA) per kg, represents the degree of lipid oxidation of foods. The results of lipid oxidation of fresh beef due to salt from kosher processing are presented in Table 5. The TBARS values of both unsalted and salted fresh beef were similar at day 0 ($P > 0.05$), but the amount of malondialdehyde from kosher beef significantly increased to three times the amount in the nonkosher beef after 7 d of storage. The increased salt content, which can lead to faster lipid oxidation, could lower the shelf life (Mariutti & Bragagnolo, 2017) and make kosher meat less desirable from salt-accelerated lipid oxidation.

The kosher process also affected the fresh beef color. Representative examples of the color of untreated control, water-soaked, and salted and rinsed beef samples are presented in Figure 1. Water soaking for 45 min caused a lighter red color (Fig. 1B) compared to the untreated control samples (Fig. 1A). After salting for 45 min and three water rinses, the color was darker with a slight brown tint (Fig. 1C) compared to the untreated control samples. This confirms that salting meat results in a loss of the red color associated with fresh meat. Hamm and Lawrie (1981) and Park et al. (1987) indicated that the addition of salt to meat destabilizes the muscle proteins, accelerates protein denaturation, and thus leads to an increased discoloration. The salting of meat has been recognized to have three effects on meat color: (1) acting as a pro-oxidant for heme pigment oxidation, causing the meat to turn brown; (2) denaturing enzymes; and (3) increasing the water-binding capacity of meat proteins, thereby making the tissues more translucent and darker (Price & Schweigert, 1987).

In conclusion, water soaking alone did not reduce the population of non-O157 STEC, *E. coli* O157:H7, and *Salmonella*. The salting processed reduced non-O157:H7, *E. coli* O157:H7, and *Salmonella* on surfaces of fresh beef, but was not enough to be considered an effective kill step. Therefore, kosher beef should be cooked to temperatures

Table 5
Effect of kosher processing on salt residues and lipid oxidation of fresh beef.

Treatment[a]	n	Storage time[b] (d)	Salt concentrations (mg/100g)	TBARS[c] (mg MDA/Kg)
Control	9	0	60.0^B	0.19^B
	9	7	59.4^B	0.32^B
Salted-rinsed	9	0	821.1^A	0.14^B
	9	7	825.6^A	0.97^A

A-B Means in the same column for each treatment with the same letter are different significantly ($P > 0.05$).
[a] Control, no treatment; Salted-rinsed, salted and rinsed, covered with kosher salt for 45 min and rinsed three times for 2 min each with water.
[b] Fresh beef samples were stored aerobically in individual plastic bags at 4°C.
[c] TBARS, thiobarbituric acid reactive substances expressed as mg malonaldehyde (MDA) per kg fresh beef samples.

Figure 1. Differences in fresh beef color during kosher processing. A, control; B, after soaking in water for 45 min; C, after salting for 45 min and rinsing.

advised by the USDA to avoid foodborne illness. The kosher salting process produced color changes and increased the salt levels of the final product, which could cause off-flavors from salt-accelerated lipid oxidation.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: NORASAK KALCHAYANAND reports administrative support was provided by USDA-ARS Roman L Hruska US Meat Animal Research Center.

Acknowledgments

The authors would like to thank Bruce Jasch for his laboratory technical support as well as Jody Gallagher for her secretarial support. The authors also would like to thank Cargill for providing kosher salt for this research study. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture (USDA). USDA is an equal-opportunity employer and provider.

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