https://doi.org/10.51483/IJAGST.1.4.2021.1-12

ISSN: 2710-3366

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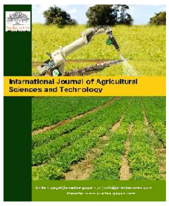

Research Paper

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Thermal Inactivation of *Salmonella* spp. Within Refrigerated or Frozen Turkey Burgers Following Pan Frying

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Article Info

Volume 1, Issue 4, November 2021
Received : 23 April 2021
Accepted : 16 September 2021
Published : 05 November 2021
doi: 10.51483/IJAGST.1.4.2021.1-12

Abstract

Turkey burgers (ca. 1.25 or 2.5 cm thick) were inoculated (ca. 6.5 log CFU/g) with a *Salmonella* spp. cocktail, stored at 4 °C (18 h) or –20 °C (30 d), and then cooked in 15 or 30 mL of canola oil. Regardless of oil volume, cooking refrigerated 1.25 cm thick burgers to 57.2, 65.6, 73.9, or 82.2 °C delivered reductions of ca. 4.8 to $\geq$ 6.0 log CFU/g compared to ca. 3.0 to $\geq$5.0 log CFU/g for frozen burgers. Cooking refrigerated 2.5 cm thick burgers to 57.2 to 82.2 °C delivered reductions of ca. 2.8 to $\geq$ 6.1 log CFU/g compared to ca. 2.4 to $\geq$5.1 log CFU/g for frozen burgers. Average internal temperatures for refrigerated or frozen burgers cooked to 57.2, 65.6, 73.9, or 82.2 °C ranged from 38.3 to 96.2, 48.0 to 99.4, 55.2 to 98.5, or 59.4 to 98.3 °C, respectively. Thus, pan frying refrigerated or frozen Turkey burgers to $\geq$73.9 °C delivered a $\geq$5.0-log reduction of *Salmonella*.

Keywords: *Salmonella, Ground turkey, Thermal inactivation, Cooking, Turkey burgers, Pan frying*

1. Introduction

Demand for poultry continues to increase worldwide (ca. 14 kg/person annually), including within the US wherein consumption was recently estimated at ca. 50 kg/person annually (OECD, 2017). As reported by Rouger *et al.* (2017),

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turkey products comprise ca. 25% of poultry meat consumed worldwide, being a distant second to chicken products which were estimated at ca. 75% of total poultry meat consumed. Poultry was also among the most common vehicles of foodborne illness reported in the US between 1998 and 2012, largely attributed to, as expected, *Campylobacter* and *Salmonella* (Chai *et al.*, 2017). In fact, active surveillance by the US Centers for Disease Control and Prevention (CDC) identified 11 outbreaks of salmonellosis over the past decade from consumption of undercooked chicken or turkey, inclusive of intact, non-intact, and comminuted products (CDC, 2021a). In addition to causing salmonellosis, another consequence of its pervasiveness in raw (ground) turkey has been several, and at times expansive, recalls of turkey products due to contamination with *Salmonella* spp. Pertinent to the present study, a notable outbreak attributed to *Salmonella* Hadar occurred in 2011 across 10 states which resulted in 12 persons being infected and ca. 55,000 lbs of frozen, raw turkey burger products being recalled (CDC, 2011a). A second outbreak, attributed to *Salmonella* Heidelberg, also occurred in 2011, this one being distributed across 34 states and leading to 136 persons becoming infected, including one death, and ca. 36.2 million lbs of raw ground turkey products being recalled (CDC, 2011b). Another outbreak (Hassan *et al*., 2019; USDA, 2018) which occurred between 2018-2019 involving *Salmonella* Reading across 42 states and the District of Columbia (DC), was linked to raw turkey products such as whole turkey, turkey pieces and ground turkey, and raw turkey pet food, as well as live turkeys handled by workers in a processing facility; there were 356 cases which resulted in 132 hospitalizations and one death, and ca. 40,000 lbs of raw pet food and ca. 300,000 lbs of raw ground turkey were recalled. Also in 2018-2019, a multistate outbreak associated with *Salmonella* Schwarzengrund resulted in seven cases of salmonellosis and one hospitalization across three states, as well as a nationwide recall of ca. 78,000 lbs of fresh raw ground turkey products (CDC, 2019 and USDA, 2019). Lastly, a multistate outbreak is currently under investigation due to the occurrence of 28 illnesses and 2 hospitalizations attributed to raw ground turkey contaminated with *Salmonella* Hadar (CDC, 2021b): no recall was issued since contaminated products are no longer available for purchase (USDA, 2021). However, a public health alert was issued for ca. 212,000 lbs of the associated raw ground turkey products, given that consumers may still have the (potentially) contaminated ground turkey stored frozen at home (USDA, 2021). Investigations of poultry-linked outbreaks identified food handling errors and inadequate cooking as the most common factors leading to poultry associated salmonellosis (Chai *et al.*, 2017).

It is widely known that raw turkey products harbor *Salmonella*. For example, cells of *Salmonella* were recovered from ca. 50% of 296 raw ground turkey samples from some 40 Federally-inspected processing plants nationwide (USDA, 1996). As another example (Cui *et al.*, 2015), over a 10-month period at a cooperating turkey abattoir a total of 300 samples/sets of matched turkey parts (i.e., neck skin, spleen, and drumsticks) from the same bird from among 20 flocks were analyzed. From among each of the 300 separate but matched turkey parts tested, cells of *Salmonella* were recovered from 42% of neck skin, 9.3% of spleen, and 6.7% of drumstick samples (Cui *et al.*, 2015). In this same study (Cui *et al.*, 2015), 14.5% of 117 raw ground turkey samples from the cooperating turkey plant also tested positive for *Salmonella:* pathogen levels were estimated at 1.9 MPN/g. Mazengia *et al.* (2014) also reported that ca. 13.3% of 180 retail ground turkey samples tested positive for *Salmonella*: pathogen levels for ground turkey, as well as for the other raw turkey products analyzed in this study, collectively ranged from 0.03 to 2.4 MPN/g. As a final example, in a retail survey of ca. 200 total samples of raw ground beef, pork, chicken, and turkey, isolates of *Salmonella* were recovered from ca. 20% of the total samples tested, including 12 of 50 turkey samples (White *et al.*, 2006).

Although *Salmonella* spp. occur naturally in poultry, at present, cells of this pathogen are not considered adulterants in raw poultry (Hibbard and Kalousi-Tatum, 2020). Due to its widespread occurrence, relatively high prevalence, and at times elevated levels in raw turkey there have been several recalls and a handful of salmonellosis outbreaks, the latter as a result of undercooking or improper handling/storage of raw turkey containing cells of *Salmonella*. Given that cooking is arguably the most effective intervention for lowering the likelihood of salmonellosis attributed to raw turkey, the objective of this study was to quantify inactivation of *Salmonella* in turkey burgers of different thicknesses, previously stored refrigerated or frozen, following pan frying in different volumes of cooking oil.

2. Materials and Methods

2.1. Bacterial Strains

The following nine-strain cocktail of genetic-marked (100 μg rifampicin/mL; TCI America, Portland, OR) strains of *Salmonella* spp. was used to inoculate raw ground turkey burgers: (i) *Salmonella* Heidelberg WA17839 (JF6X01.0122, clinical isolate); (ii) *Salmonella* Heidelberg WA17853 (JF6X01.0122, food isolate); (iii) *Salmonella* Heidelberg 2011K-1224 (JF6X01.0058, ground turkey isolate); (iv) *Salmonella* Hadar CO-2955; (v) *Salmonella* Heidelberg FY-14-6 (JF6X01.0045, chicken parts isolate); (vi) *Salmonella* FSIS Control 1; (vii) *Salmonella* FSIS Control 2; (viii) *Salmonella*

FSIS 38; and (ix) *Salmonella* FSIS 39. Each strain was maintained and subsequently prepared as a cocktail for this study as previously described (Porto-Fett *et al.*, 2008, 2019; and Luchansky *et al.*, 2020).

2.2. Inoculation and Cooking of Ground Turkey

Fresh ground turkey breast was purchased directly from a local producer (Koch's Turkey Farm, Tamaqua, PA) and stored at –20 °C until used. Results of the proximate compositional analyses of the ground turkey breast (a 500-g composited portion from each of three trials; N = 1, n = 3), as conducted by a private testing laboratory according to methods approved and described by the Association of Official Analytical Chemists (AOAC, 2012), were as follows: 0.6 ± 0.46% fat, 72.3 ± 0.25% moisture, 25.7 ± 0.25% protein, pH 5.67 ± 0.24, and a_w 0.990 ± 0.001. The meat was inoculated, formulated, shaped, cooked, and sampled using methods described in our previous studies (Duong *et al.*, 2020; and Luchansky *et al.*, 2013, 2019, 2020). In brief, ground turkey (ca. 13 kg) was inoculated with 130 mL of the *Salmonella* cocktail to achieve a target level of ca. 6.5 log CFU/g of meat. The inoculated turkey was combined with a spice mixture comprised of salt (1.42%; SaltWorks Inc., Woodinville, WA), black pepper (0.45%; The Sausage Maker, Buffalo, NY), garlic powder (0.45%; The Sausage Maker), and onion powder (0.45%; The Sausage Maker), and then mechanically mixed for ca. 2 min in a commercial countertop mixer (Univex SRM12; Univex, Salem, NH) at room temperature (21 ± 1 °C). Portions of the ground turkey mixture were placed onto a sheet of dry waxed paper (14 x 14 cm; Cabela's, Sidney, NE) within a non-stick press (model UK-516023; Cabela's) to subsequently form patties of uniform thickness [ca. 1.25 cm (ca. 122 g) or ca. 2.5 cm (ca. 255 g)] and diameter (ca. 10.5 cm). Burgers were placed onto sterile Styrofoam trays (1012S; Genpak, Glens Falls, NY) that were subsequently inserted individually into nylon-polyethylene bags (Koch Supplies, Kansas City, MO). After heat sealing, packages of burgers were then held at 4 °C for up to 18 h or at –20 °C for up to 30 d since both "types/states" of turkey burgers are available for purchase at food retailers. Prior to freezing, a single Type J thermocouple (model 304-J-MO 127 125; Omega Engineering, Stamford, CT) was inserted from the side of each burger into the approximate geometric center. Otherwise similar burgers, inoculated but not cooked, were used as controls.

The inoculated ground turkey burgers were cooked to target internal temperatures of 57.2 °C (135°F), 65.6 °C (150°F), 73.9 °C (165°F), or 82.2 °C (180°F), in a non-stick sauté pan (30.5 cm diameter x 4 cm depth; Tefal Professional E9380894; Rumilly, Haute-Savoie, France) containing canola oil (15 mL or 30 mL; Refined Expeller Pressed, Whole Foods Market, Austin, TX) on a countertop induction burner (Duxtop 8100MC; Secura, Brookfield, WI) maintained at ca. 175 °C (ca. 350 °F; medium-high heat) as per the instructions on the product label. Burgers were pan fried based on information collected from the labels of 28 packages of ground turkey purchased at retail (see below). For practical purposes there was no appreciable difference in depth of oil (ca. 0.2 mm depth) whether 15 or 30 mL of oil were added to the pan. A single thermocouple (Type J) was inserted into the approximate geometric centre from the side of each burger. An additional Type J thermocouple was used to record the surface temperature of the pan itself. All temperatures were monitored continuously in 5 s intervals via thermocouples connected to an eight-channel thermocouple logger (model OM-CP-OCTTEMP; Omega).

As for our related studies using beef- and plant-based products (Luchansky *et al.*, 2020), burgers were flipped every ca. 2 min with the aid of two stainless steel spatulas until the target internal temperature was achieved. Periodic flipping of burgers presumably facilitates more uniform heat transfer and lessens the likelihood that the surface of a burger would be overdone (i.e., charred, crusted, or burned). Once cooked, burgers were placed onto a polystyrene foam packaging tray (Koch Supplies, Kansas City, MO) and weighed. Within ca. 15 s after weighing, up to eight additional temperatures were recorded using a handheld Type K digital thermocouple (Model AquaTuff Waterproof 351 Digital Thermocouple; Cooper Atkins, Middlefield, CT) to chronicle temperature variations at several locations within each burger as soon as possible after cooking. Doing so enabled reporting of attendant temperature ranges and averages for a specific endpoint temperature, as well as the identification of potential cold spots within each cooked burger. Next, cooked burgers were transferred into filtered stomacher bags (Type XX-C003; Microbiology International, Frederick, MD), placed on ice, and then sampled within 30 min for surviving *Salmonella* cells as described (Luchansky *et al.*, 2020).

2.3. Microbiological Analyses of Inoculated Burgers After Cooking

Salmonella were enumerated from uncooked and cooked burgers by macerating each less thick burger (ca. 1.25 cm; ca. 95 g after cooking) in 100 mL and each thicker burger (ca. 2.5 cm; ca. 210 g after cooking) in 200 mL of sterile 0.1% peptone (Difco; Becton, Dickinson Company, Sparks, MD) water as described (Luchansky *et al.*, 2020). Previous studies by our group (Calle *et al.*, 2015; Data not shown) established that some (heat injured) cells of *Salmonella* were not recoverable on XLT4 agar in comparison to TSA agar; however, this difference was not appreciable (<0.5 log CFU/g). In addition, when pathogen levels decreased to below a detection limit by direct plating, for statistical analyses (see below) of these data, the detection limit of <0.34 logCFU/g was used as the log CFU/g value for both positive and negative enrichments as described (Ihnot *et al.*, 1998).

2.4. Survey of poultry burgers available at retail

For the purpose of this study, an informal, non-randomized survey was conducted at food retailers within the vicinity of Raleigh, NC, to collect general information about the appliance used and recommended endpoint temperature for cooking turkey burgers. Another survey was conducted at food retailers in PA an NJ within the vicinity of Wyndmoor, PA, to collect general information about the state of burgers (i.e., refrigerated versus frozen), types of packaging, label information (i.e., handling and cooking instructions, list of ingredients), and the size and formulation of poultry burgers available for purchase. As part of the NC survey, a total of 28 samples of ground turkey were purchased. For the PA and NJ survey, a total of 36 samples/burgers (18 brands), that being 29 ground turkey and seven ground chicken burgers, were purchased at 10 large chain stores and two specialty stores/meat markets during four shopping trips over a two-week period in Spring of 2017. Stores were selected based on convenience and proximity to our laboratories, and because the stores selected provided good representation of locations from where a considerable number of consumers would purchase turkey burgers.

2.5. Statistical analyses

Means and standard deviations were calculated from data for each trial using the Microsoft Excel 2010 software (Redmond, WA). A total of 228 cooked (i.e., experimental) and 18 uncooked (i.e., control) burgers were analyzed from among three trials and three replicates per experimental treatment (N=3, n=3). The SAS system (SAS Version 9.4; SAS Institute, Cary, NC) was used to analyze and determine statistical significance among the following factors: volume of oil, burger weight/thickness, state of burger prior to cooking, and cooking temperatures. Analysis of Variance (ANOVA) was used to determine the effects and interactions of the abovementioned factors on the log reduction values using the PROC MIXED procedure. Differences in lethality observed for cooking oil volume, burger weight/thickness, state of the burger prior to cooking, and cooking temperatures and/or combinations thereof were considered as significant using the Sidak method at the $p = 0.05$ significance level.

3. Results

3.1. Survey of Poultry Burgers Available at Retail

Regarding our survey of turkey burgers available for purchase at food retailers in NC, more labels recommended that burgers should be pan fried rather than grilled or broiled (Data not shown). Regarding our survey of turkey burgers available for purchase at food retailers in PA and NJ, most of the burgers (80.6%; 29 of 36) were pre-formed by processors, whereas 19.4% (7 of 36) of the burgers were formed at the store. None of the burgers were breaded, and none of the burgers were ready-to-eat. For pre-formed burgers, 72.4% (21 of 29) of the samples were sold frozen and 27.6% (8 of 29) were sold refrigerated. However, for burgers that were formed at the store, most (85.7%; 6 of 7) were sold as refrigerated; only one sample was sold as frozen. Regarding packaging, 5.6% (2 of 36) of the samples were hand-wrapped in butcher paper, 41.7% (15 of 36) were overwrapped with film on a foam tray, and 52.8% (19 of 36) were packaged on a plastic tray that was wrapped with a plastic film under a modified atmosphere. Most burgers were round (86.1%; 31 of 36), followed by burgers with “flower” (8.3%; 3 of 36) and “oval” (5.6%; 2 of 36) shapes. The average fat content and weight as listed on the label were 8.0 ± 2.6 g (ranging from 3.6 to 14.1 g) and 132.9 ± 29.2 g (ranging from 83.5 to 214 g), respectively, whereas the diameter and thickness of burgers were 10.4 ± 1.1 cm (ranging from 7.5 to 12.5 cm) and 1.25 ± 0.4 cm (ranging from ca. 0.5 to 2.0 cm), respectively.

All ground chicken and ground turkey burgers displayed safe handling instructions on the package label. Examples of suggested safe handling instructions were: (i) “some food products may contain bacteria that could cause illness if the product is mishandled or cooked improperly”; (ii) “for your protection, follow these safe handling instructions: keep refrigerated or frozen”; (iii) “thaw in refrigerator or microwave”; (iv) “keep raw meat and poultry separate from other foods”; (v) “wash working surfaces including cutting boards, utensils and hands after touching raw meat or poultry”; and (vi) “cook thoroughly”. Regarding cooking instructions/recommendations, only four of the 36 samples (11.1%) did not display any cooking recommendations, whereas 88.9% (32 of 36) recommended to: (i) cook burgers thoroughly to a specified internal temperature such as 165 °F (69.4%; 25 of 32 samples) or 170 °F (12.5%; 4 of 32 samples); (ii) cook burgers thoroughly, but did not state a target internal temperature (3.1%; 1 of 32 samples); (iii) cook by time per side

(3.1%; 1 of 32 samples); or (iv) cook until browned on both sides and to "desired doneness" in the middle (2.9%; 1 of 32 samples). Of the 36 burgers surveyed, 13.9% (5 of 36) did not have any ingredients listed on the label. For those burgers that had ingredients listed on the label, the most common ingredients added to the formulation of chicken and turkey burgers were: salt (71%, 22 of 31), rosemary extract (58.1%; 18 of 31), natural flavoring (48.4%; 15 of 31), garlic powder (35.5%, 11 of 31), and/or onion powder (32.3%, 10 of 31). Some burgers also contained ingredients that imparted functionality such as flavor enhancers (e.g., hydrolyzed corn protein, autolyzed yeast extract, or corn syrup solids), vitamins (e.g., thiamine hydrochloride), preservatives (e.g., lactic acid or sorbic acid), or binding agents (e.g., bread crumbs, potassium or sodium phosphate, milk protein, or sodium bicarbonate).

3.2. Cooking times and cooking temperatures

Overall, the times required to achieve the target internal temperatures of 57.2, 65.6, 73.9, or 82.2 °C were significantly longer ($p < 0.05$) for frozen turkey burgers when compared to refrigerated burgers. In addition, regardless of the initial state of the burgers (i.e., refrigerated or frozen) or volume of oil (i.e., 15 or 30 mL), the cooking times required to achieve the target internal temperatures within 2.5 cm thick burgers were significantly longer ($p < 0.05$) when compared to results for otherwise similar burgers that were 1.25 cm thick. Also, the higher the internal target temperature, the longer ($p < 0.05$) the time needed for cooking either refrigerated and frozen burgers. Irrespective of the volume of oil used, the time required for cooking refrigerated burgers to internal temperatures of 57.2 °C to 82.2 °C ranged from 4.9 to 8.0 min for 1.25 cm thick burgers compared with 12.2 to 20.3 min for 2.5 cm thick burgers. For frozen burgers, the time required for achieving the target internal temperatures ranged from 7.0 to 12.7 min (1.25 cm thick) to 20.8 to 31.9 min (2.5 cm thick).

Regarding the variability and range of cooking temperatures achieved within turkey burgers (Table 1), there was no association between the volume of oil, thickness of burgers, or state of burgers prior to cooking and the internal burger temperatures. Irrespective of the volume of cooking oil used, when 1.25 cm thick burgers, previously stored refrigerated, were cooked to internal temperatures of 57.2, 65.6, 73.9, or 82.2 °C, the average final internal temperature readings were 67.5 ± 8.5 °C, 77.2 ± 11.2 °C, 80.5 ± 7.7 °C, or 86.5 ± 6.3 °C, respectively. When 2.5 cm thick burgers, previously stored refrigerated, were cooked to 57.2, 65.6, 73.9, or 82.2°C, the average final internal temperatures were 67.2 ± 11.9 °C, 75.2 ± 11.2 °C, 80.2 ± 8.4 °C, or 85.7 ± 6.1 °C, respectively. Likewise, the average final internal temperatures within frozen burgers formed to a thickness of 1.25 cm and pan fried in 15 or 30 mL of canola oil to target temperatures of 57.2, 65.6, 73.9, or 82.2 °C were 61.6 ± 5.3 °C, 67.2 ± 5.3 °C, 74.3 ± 5.8 °C, or 81.8 ± 5.6 °C, respectively. For frozen burgers formed to a thickness of 2.5 cm and irrespective of cooking oil volume, the average final internal temperatures following cooking to 57.2, 65.6, 73.9, or 82.2 °C were 61.6 ± 7.1 °C, 67.3 ± 7.2 °C, 75.8 ± 5.9 °C, or 81.6 ± 5.2 °C, respectively.

Table 1: Internal Temperatures in Fresh and Frozen Turkey Burgers Cooked to Endpoint Temperatures of 57.2, 65.6, 73.9, or 82.2 °C (N = 3, n = 3)

Thickness (cm)	Volume of Oil (mL)	Target End Point Temperature (°C)			
		57.2	65.6	73.9	82.2
1.25	15	66.3 ± 7.3[1,2] (49.2 – 80.8)[3]	77.6 ± 8.4 (55.5 – 95.8)	80.1 ± 8.3 (56.8 – 95.5)	87.2 ± 6.0 (65.5 – 97.8)
	30	68.8 ± 9.4 (47.2 – 95.5)	76.7 ± 26.2 (56.8 – 99.4)	80.7 ± 7.1 (64.9 – 98.5)	86.1 ± 5.94 (73 – 99.4)
2.5	15	68.4 ± 12.0 (53.3 – 94.5)	74.2 ± 11.6 (51.1 – 96.6)	80.8 ± 8.6 (55.5 – 98.4)	85.8 ± 6.5 (62.2 – 98.3)
	30	65.9 ± 11.7 (50.2 – 96.2)	76.1 ± 10.9 (49.6 – 98.6)	79.7 ± 8.2 (55.2 – 96.9)	85.3 ± 6.2 (74.5 – 100.1)

Note: [1]For a given temperature, thickness of burger, volume of cooking oil, and for both refrigerated and frozen burgers, the internal temperatures reported represent the average of the target temperature achieved while burgers were in the frying pan as determined via a Type J thermocouple plus 8 additional temperatures within each burger that were taken using a hand-held Type K digital thermocouple immediately after burgers were removed from the frying pan.
[2]Mean ± standard deviation.
[3]Range of minimum and maximum internal temperatures.

3.3. Thermal Inactivation of Salmonella spp. in Turkey Burgers

For a given cooking temperature, state of meat, or burger thickness, with the exception of 2.5 cm thick refrigerated burgers cooked to 82.2 °C, there was no significant ($p > 0.05$) effect of the volume of cooking oil on thermal inactivation of *Salmonella*. Regarding the effect of the state of meat on inactivation of *Salmonella*, with the exception of 1.25 cm thick burgers that were cooked to 57.2 °C in 15 mL of oil or cooked to 65.6 °C in 30 mL of oil, significantly ($p < 0.05$) more cells of *Salmonella* were inactivated after cooking refrigerated burgers when compared to frozen burgers. Likewise, for 2.5 cm thick burgers, significantly ($p < 0.05$) more cells of *Salmonella* were inactivated in refrigerated burgers when compared to frozen burgers that were cooked to 65.6, 73.9, or 82.2 °C in 30 mL of oil. However, no significant ($p > 0.05$) differences in pathogen lethality were observed between 2.5 cm thick refrigerated or frozen burgers that were cooked to 57.2, 65.6, 73.9, or 82.2 °C in 15 mL of oil or to 57.2 °C in 30 mL of oil.

Regarding the effect of burger thickness on pathogen inactivation, significantly ($p < 0.05$) more cells of *Salmonella* were inactivated in previously refrigerated 1.25 cm thick burgers cooked to 57.2 or 65.6 °C in 15 mL of oil or that were cooked to 57.2 °C in 30 mL of oil when compared to previously refrigerated 2.5 cm thick burgers cooked to the same endpoint temperatures and in the same volume of oil. Similarly, significantly ($p < 0.05$) more cells of *Salmonella* were inactivated in previously frozen 1.25 cm thick burgers cooked to 65.6 °C in 30 mL of oil when compared to previously frozen 2.5 cm thick burgers cooked to the same endpoint temperature and in the same volume of oil. Lastly, for 1.25 cm thick burgers, cooking temperatures of 65.6, 73.9, or 82.2 °C were significantly ($p < 0.05$) more effective at inactivating the pathogen than cooking to an endpoint temperature of 57.2 °C for refrigerated burgers cooked in 15 mL of oil or in frozen burgers cooked in 30 mL of oil. In addition, no statistical differences ($p > 0.05$) were noted for thermal inactivation of *Salmonella* in previously refrigerated 1.25 cm thick burgers that were cooked in 30 mL of oil to 57.2, 65.6, or 82.2 °C when compared with otherwise similar burgers cooked to 73.9 °C. Similarly, no statistical differences ($p > 0.05$) were noted for thermal inactivation of *Salmonella* in previously frozen 1.25 cm thick burgers cooked in 15 mL of oil that were cooked to 57.2 °C when compared with otherwise similar burgers cooked to 65.6 °C. However, significant differences ($p > 0.05$) were noted when frozen 1.25 cm thick burgers cooked to 57.2 °C in 15 mL of oil were compared with similar burgers that were cooked to 73.9 and 82.2 °C. No statistical differences ($p > 0.05$) were noted for thermal inactivation of *Salmonella* among previously frozen 1.25 cm thick burgers cooked in 15 mL of oil that were cooked to 65.6 °C when compared with otherwise similar burgers cooked to 73.9 or 82.2 °C. Likewise, for 2.5 cm thick burgers, stored either refrigerated or frozen, that were cooked in 15 mL of cooking oil or frozen 2.5 cm thick burgers that were cooked in 30 mL of oil, significantly ($p < 0.05$) more cells of *Salmonella* were inactivated at temperatures of 73.9 or 82.2 °C when compared to endpoint temperatures of 57.2 or 65.6 °C. For previously refrigerated 2.5 cm thick burgers that were cooked in 30 mL of oil, significantly ($p < 0.05$) more cells of *Salmonella* were inactivated at endpoint temperatures of 65.6, 73.9, or 82.2 °C when compared to 57.2 °C, albeit with cooking to an end point temperature of 82.2 °C being significantly ($p < 0.05$) more effective at inactivating the pathogen than cooking to endpoint temperatures of 65.6 or 73.9 °C.

Irrespective of the volume of oil, state of the meat prior to cooking, or weight/thickness of the burger, pan frying inoculated turkey burgers to target internal temperatures of 73.9 or 82.2 °C resulted in total reductions of resulted in >5.0 log CFU/g in *Salmonella* numbers. When cooking to an internal temperature of 65.6 °C, and irrespective of oil volume, total reductions of >5.6 log CFU/g were also achieved for 1.25 cm thick burgers that were stored refrigerated prior to cooking. In contrast, when otherwise similar but thicker (2.5 cm) burgers, previously stored refrigerated, were cooked to 65.6 °C in 15 or 30 mL of oil, pathogen numbers were reduced by ca. 3.7 or 4.9 log CFU/g, respectively. Likewise, regardless of oil volume or burger thickness, pan frying frozen turkey burgers to an internal temperature of 65.6 °C resulted in reductions of ca. 3.4 to 4.6 log CFU/g in *Salmonella* numbers. When cooking to an internal temperature of 57.2 °C, and irrespective of oil volume, total reductions of ca. 4.8 log CFU/g were achieved for 1.25 cm thick burgers that were stored refrigerated prior to cooking. However, when otherwise similar but thicker (2.5 cm) burgers, previously stored refrigerated, were cooked to 57.2 °C in 15 or 30 mL of oil, pathogen numbers were reduced by ca. 2.8 log CFU/g. Lastly, irrespective of thickness, total reductions of ca. 2.4 to 3.2 log CFU/g in pathogen numbers were observed when previously frozen burgers were pan fried to 57.2 °C in 15 or 30 mL of canola oil.

For refrigerated burgers, irrespective of cooking oil volume or burger thickness, it was not possible to recover cells of *Salmonella* by direct plating or by enrichment from 86.9% (33 of 38 burgers) and 81.6% (31 of 38 burgers) of burgers cooked to 73.9 and 82.2 °C, respectively (Data not shown). In contrast, for burgers previously refrigerated and cooked to 57.2 or 65.6 °C cells of *Salmonella* were not recovered by direct plating or by enrichment from 13.9% (5 of 36 burgers) or 48.7% (19 of 39 burgers) of burgers, respectively (Data not shown). Likewise, irrespective of cooking oil volume or burger thickness, when frozen burgers were cooked to 73.9 or 82.2 °C, cells of *Salmonella* were not recovered by direct plating or by enrichment from 83.3% (30 of 36 burgers) or 81.6% (31 of 38 burgers) of burgers, respectively. Lastly, the pathogen was not recovered by direct plating or by enrichment from 20% (8 of 40 burgers) and 42.5% (17 of 40 burgers) of previously frozen turkey burgers subsequently cooked to 57.2 and 65.6 °C, respectively.

As assessed empirically, and regardless of the volume of oil, thickness of burgers, or storage temperature of burgers prior to cooking, when turkey burgers were cooked to an internal temperature of 73.9 or 82.2 °C all nine burgers per each treatment displayed the characteristic "white color" expected for a fully-cooked poultry burger. Likewise, no pinkish spots were observed in 1.25 cm thick burgers stored refrigerated or frozen and subsequently cooked in 15 or 30 mL of oil to an internal temperature of 65.6 °C. However, for 2.5 cm thick burgers stored refrigerated and subsequently cooked to an internal temperature of 65.6 °C in 15 or 30 mL of oil, 66.7% (6 of 9) or 27% (3 of 11) of burgers, respectively, displayed pink/pinkish spots throughout the burger. For 1.25 cm thick burgers stored refrigerated and subsequently cooked in 15 mL of oil to an internal temperature of 57.2 °C, all nine burgers displayed pink/pinkish spots throughout, whereas for otherwise similar burgers cooked in 30 mL of oil only 33.3% (3 of 9) displayed pink/pinkish spots. Lastly, for 2.5 cm thick burgers cooked to an internal temperature of 57.2 °C, and regardless of the volume of oil or storage temperature, all nine burgers displayed pink/pinkish spots.

4. Discussion

Much is commonly known and much has been published in scientific journals on the formulation, nutritional and health benefits, sensory/quality attributes, and handling/safety of beef burgers, whereas related information on turkey burgers is lacking, especially as it relates to the behavior and persistence of microbial pathogens. In a ranking of the health burden of the top 50 pathogen-food combinations based on both cost of illness and loss of Quality Adjusted Life Years (QALYs), Batz *et al.* (2012) ranked "*Campylobacte*r-poultry" and "*S. enterica*-poultry" as first and fourth, respectively, on this list. Nonetheless, there has been a continued increase in consumer demand for poultry, in general, due its preferred taste, texture, and/or presumed health and nutritional benefits over other protein sources, notably its beef counterpart, with chicken being consumed in 2020 at ca. 95 lbs per capita in the US and with beef coming in second and being consumed at ca. 58 lbs (Wong *et al.*, 1993; and Leake, 2021). An unintended consequence of poultry's increased popularity has been a concomitant increase in the price of ground turkey, typically making it more costly than ground beef (Munoz, 2021). Thus, additional studies are warranted to elaborate the time and temperature combinations required to deliver an appreciable reduction of cells of *Salmonella* during cooking of turkey-based burgers.

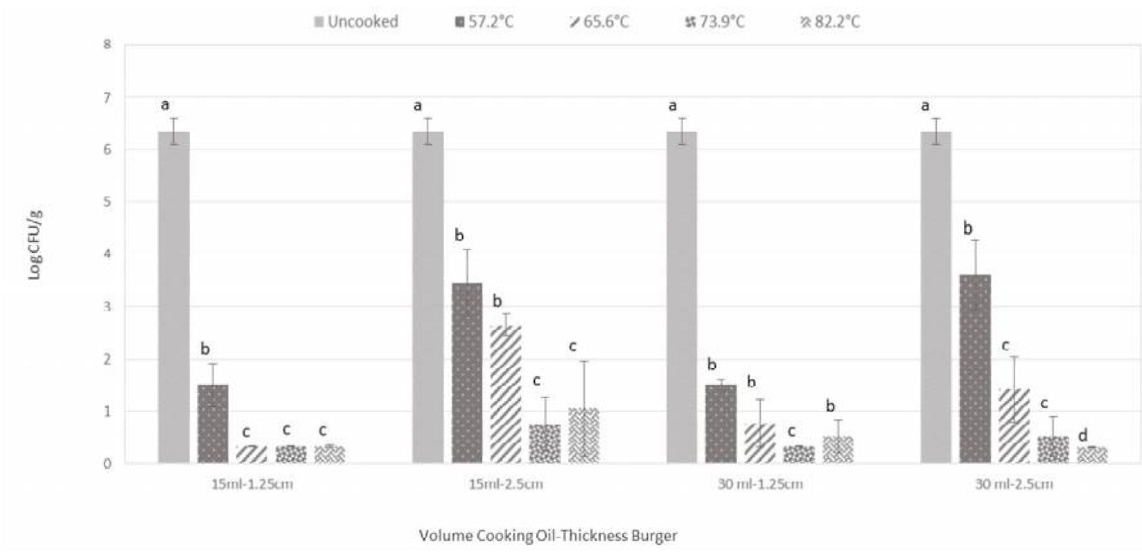

Figure 1: Thermal inactivation of *Salmonella* in refrigerated ground turkey burgers after being pan fried in 15 or 30 mL of canola oil to internal temperatures of 57.2, 65.6, 73.9 or 82.2 °C (N = 3 Trials, n = 3 burgers per trial per temperature per treatment). Error bars represent the standard deviation of the mean. For a given volume of cooking oil and burger thickness, means with different lower-case letters denote significant ($p \leq 0.05$) differences among endpoint cooking temperatures with respect to pathogen inactivation.

We quantified the fate of *Salmonella* spp. in turkey burgers following pan frying to temperatures ranging from 57.6 to 82.2 °C. Parameters evaluated in addition to cooking temperatures included the state of the burgers just prior to cooking (i.e., refrigerated or frozen), the volume of cooking oil added to the frying pan, and the thickness of each burger. As is evident by comparing the data in Figures 1 and 2, there was a significant ($p < 0.05$) difference in the initial levels of *Salmonella* between uncooked burgers stored refrigerated (4 °C) versus stored frozen (–20 °C): pathogen levels in refrigerated burgers just prior to cooking were ca. 6.3 ± 0.25 log CFU/g, whereas in frozen burgers levels of *Salmonella* just prior to cooking were ca. 5.3 ± 0.29 log CFU/g. Dominguez and Schaffner (2009) reported that levels of *Salmonella* remained relatively constant in chicken nuggets and chicken strips during storage at –20 °C for up to 112 d. Manios and

Skandamis (2015) also reported that storing ground beef patties (20% fat) for 5 d at –22 °C resulted in only a ca. 0.7 log CFU/g decrease in *Salmonella* levels, with no additional reduction in pathogen numbers after 75 d. As a final example, Dykes and Moorhead (2001) reported that *Salmonella* viability was not appreciably affected when beef trimmings were held frozen at –18° or –35 °C for up 270 d. Given these nominal reductions in pathogen levels due to freezing as observed herein and reported elsewhere, neither the rate nor the effect of freezing on viability of *Salmonella* over 30 d of frozen storage were monitored herein. Regardless, the comparative reductions in levels of *Salmonella* were based on differences in the initial levels of the pathogen that were quantified in uncooked burgers (refrigerated or frozen) compared to remaining levels after cooking. As such, it is possible to directly compare the resulting lethality of pan frying refrigerated (Figure 1) and frozen (Figure 2) turkey burgers to internal temperatures of 57.6, 65.6, 73.9, or 82.2 °C on viability of *Salmonella*.

It is well established that cooking is the most effective consumer practice to eliminate foodborne pathogens that at times may be present in raw meat and poultry products. The USDA FSIS recommends that consumers cook beef burgers to 71.1 °C (160 °F) to eliminate pathogens (USDA, 2012), whereas turkey burgers must be cooked to an instantaneous internal temperature of at least 73.9 °C (165 °F) to eliminate the relatively low levels of *Salmonella* that on occasion may be present (USDA, 2013, 2020). As detailed herein and elsewhere (Routh *et al.*, 2015; Chai *et al.*, 2017; CDC, 2019; and Hassan *et al.*, 2019), over the past decade or so there have been several recalls and illnesses attributed to consumption of (undercooked and/or improperly handled) ground chicken and turkey due to contamination with cells of *Salmonella*. The associated integrated risk of public health is exacerbated because *Salmonella* are somewhat tolerant of heat, with select strains being capable of surviving some thermal processing (Matthews *et al.*, 2017). Numerous studies have reported on thermal resistance (i.e., D-values) of *Salmonella* in poultry products such as ground turkey breast and thighs and ground chicken breast and skin, as well as for chicken burgers or chicken tenders (Juneja *et al.*, 2001; Mazzotta, 2000; Murphy *et al.*, 2000, 2002, 2004a, 2004b; Takhar *et al.*, 2009; Tuntivanich *et al.*, 2008; Veeramuthu *et al.*, 1998). For example, D-values of 43.1 to 0.096, 43.8 to 0.07, and 43.3 to 0.09 min were reported for ground turkey (ca. 5.4% fat; 5-g each at ca. 1 mm thick), chicken thigh/leg meat (ca. 10.3% fat; 10-g each at ca. 1 mm thick), and chicken skin (ca. 47.4% fat; ca. 5-g each at ca. 1 mm thick), respectively, inoculated with *Salmonella* and placed within a vacuum-sealed plastic bag that was subsequently cooked to 55 to 70 °C in a circulating water bath (Murphy *et al.*, 2004a and 2004b). Mazzota (2000) also reported D-values of 3.2 to 0.18 min for *Salmonella* inoculated into ground chicken breast (5-g each at ca. 0.5 mm thick) heated to 56 to 63 °C in a thermostatically-controlled water bath. With the exception of a handful of publications (Baker *et al.*, 1983; Luchansky *et al.*, 2020; Matthews *et al.*, 2017; Murphy *et al.*, 1999 and 2001), thermal inactivation studies of *Salmonella* directly in burgers have been almost exclusively conducted using ground beef or ground chicken. For example, reductions of ca. 4.0 to > 6.0 log CFU/g in levels of *Salmonella* in ground beef burgers were observed when cooked to >71.1 °C (Luchansky *et al.*, 2020; Matthews *et al.*, 2017), whereas reductions of ca. 3.0 to >6.0 log CFU/g were observed for ground chicken burgers cooked to endpoint temperatures of >70 °C (Baker *et al.*, 1983; Murphy *et al.*, 1999, 2001; Matthews *et al.*, 2017). In a related study (Luchansky *et al.*, 2020), we demonstrated that pan frying plant-based (15.6% fat) versus beef-based (16.2% fat) burgers in 15 mL of canola oil to internal temperatures of 62.8, 68.3, or 73.9 °C decreased *Salmonella* numbers by ca. 3.5, 5.4, or 6.5 log CFU/g, respectively.

Our results validated that cooking inoculated turkey burgers (<1% fat) on a non-stick frying pan to the recommended internal temperature of >73.9 °C (USDA, 2013, 2020), irrespective of the cooking oil volumes or burger thicknesses tested, delivered a>5.0 log reduction in numbers of *Salmonella*. It should be noted that levels of surviving *Salmonella* were likely underestimated, since XLT4 is a particularly harsh recovery medium: the diversity and high levels of the indigenous background flora within raw ground turkey necessitated the use of XLT4 agar plus rifampicin to recover (surviving) cells of *Salmonella* comprising the pathogen cocktail that was inoculated into burgers. That being said, samples were enriched to assess the presence/absence of surviving cells of *Salmonella*. Our results are in general agreement with previous studies reporting on the efficacy of consumer cooking practices for inactivation of *Salmonella* in burgers prepared from turkey. For example, Murphy *etal.* (2004a) validated that a 7.0-log reduction in *Salmonella* numbers was achieved when turkey burgers (ca. 5.5% fat; ca. 1.3 cm thick and ca. 12.7 cm diameter) were cooked to an internal temperature of 70 °C in an air impingement oven set at 232 °C, with an air velocity of 0.02 m/s and 50% air humidity. Roccato *et al.* (2015) reported that pan frying turkey burgers (ca. 105 g, 1.3 cm thick and ca. 9.5 cm diameter) for 5 or 10 min in 25 mL of olive oil decreased *Salmonella* numbers to below detection (<1.3 MPN/g) from initial inoculation levels of 1.0 to 3.0 log CFU/g.

Temperature variability within burgers during cooking may be attributed to several factors including the moisture and fat content, formulation (e.g., inclusion of non-meat ingredients such as mushrooms, bacon, grated cheese, etc.), state of the meat prior to cooking (e.g., refrigerated or frozen), weight, diameter, and thickness of burgers, number of times burgers are flipped (e.g., single or multiple), and cooking method (e.g., grilled, sautéd, baked, etc.) which, in turn, may have a direct effect on pathogen inactivation (Doyle and Mazzota, 2000; Luchansky *et al.*, 2020, 2013; Mattick *et al.*, 2002; Porto-Fett *et al.*, 2016; Smith *et al.*, 2001; and Tuntivanich *et al.*, 2008). Given the range and variation in temperatures we observed for a given target endpoint cooking temperature, as well as the attendant recovery of sporadic survivors due to presumed cold spots within the meat, our findings also confirm the importance of proper and frequent thermometer use to determine doneness. Further studies are warranted or underway to evaluate additional parameters that could appreciably impact on thermal inactivation of *Salmonella* within turkey burgers in response to heat, including, but not limited to, strain-to-strain variation, use of pathogen cocktails versus single strains, effect of cooking appliances, and flipping of burgers, as well as burger shape, density, and waffling. In the interim, as reported herein we validated that the USDA FSIS recommended minimum endpoint internal temperature of 73.9 °C (165 °F) for cooking ground poultry, as measured with a food thermometer (USDA, 2013; and 2020), can appreciably lower levels of *Salmonella* and significantly lessen the risk of salmonellosis associated with turkey burgers.

Disclosure: Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture (USDA). The USDA is an equal opportunity provider and employer.

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Cite this article as: Minh Duong, Anna, C.S. Porto-Fett, Bradley A Shoyer, Yangjin Jung, Laura, E. Shane, Manuela Osoria, Elizabeth Henry, Benjamin, J. Chapman and John, B. Luchansky (2021). Thermal Inactivation of *Salmonella* spp. Within Refrigerated or Frozen Turkey Burgers Following Pan Frying. *International Journal of Agricultural Sciences and Technology.* 1(4), 1-12. doi: 10.51483/IJAGST.1.4.2021.1-12.