

Effects of Domestic Storage and Thawing Practices on *Salmonella* in Poultry-Based Meat Preparations

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ABSTRACT

Among consumer food handling practices, time-temperature abuse has been reported as one of the most common contributory factors in salmonellosis outbreaks where the evidence is strong. The present study performed storage tests of burgers, sausages, and kebabs and investigated (i) the effect of refrigerator temperatures (4°C versus 8 or 12°C, which were the temperatures recorded in 33 and 3%, respectively, of domestic refrigerators in Italy), with or without prior temperature abuse (25°C for 2 h, simulating transport of meats from shop to home), and (ii) the impact of the thawing method (overnight in the refrigerator at 8°C versus on the kitchen countertop at 23°C) on the presence and numbers of *Salmonella* bacteria. Storage tests were carried out on naturally or artificially (*Salmonella enterica* serovar Typhimurium at ca. 10 CFU/g) contaminated products, while freezing-thawing tests were conducted only on artificially contaminated products (*Salmonella* Typhimurium at ca. 10, 100, and 1,000 CFU/g). The results from the artificially contaminated products showed significant ($P < 0.05$) growth of *Salmonella* Typhimurium at 12°C (i.e., from ca. 8 most probable number [MPN]/g to >710 MPN/g) in kebabs after 7 and 10 days but more moderate growth in sausages (i.e., from ca. 14 MPN/g to a maximum of 96 MPN/g after 9 days of storage). Storage of naturally contaminated burgers or sausages (contamination at or below 1 MPN/g) at 4, 8, or 12°C and a short time of temperature abuse (2 h at 25°C) did not facilitate an increase in the presence and numbers of *Salmonella* bacteria. Thawing overnight in the refrigerator led to either a moderate reduction or no change of *Salmonella* Typhimurium numbers in burgers, sausages, and kebabs. Overall, this study showed that domestic storage and thawing practices can affect food safety and that time-temperature abuse can cause a substantial increase of *Salmonella* numbers in some types of poultry-based meat preparations, highlighting that efforts for the dissemination of consumer guidelines on the correct storage and handling of meats need to be continued.

Reduction of the incidence of foodborne diseases and governing food safety remain important priorities both for food industries and for competent authorities because of the consequences of such diseases on public health, the loss of consumer trust in the food supply chain, and the economic impact on both food producers and society. *Salmonella* bacteria remain the most frequently reported cause of foodborne outbreaks in the European Union, accounting for 28.6% of all outbreaks and 45.5% of outbreaks with strong evidence in 2012 (15). Source attribution of human salmonellosis identified pigs as the main source in Italy, accounting for 43 to 60% of infections, followed by poultry (18 to 34%) (44). At the European level, the laying hen reservoir was estimated to be the most important source, contributing 43.8% of cases, followed by pigs (26.9%). Turkeys and broilers were estimated to be less important sources of *Salmonella*, contributing 4.0 and 3.4% of cases, respectively (51).

In the European Union, 58% of *Salmonella* outbreaks with strong evidence were traced to foods consumed at home (15). Nevertheless, surveys on risk perception in European consumers demonstrated that they are very confident about being able to personally take action to avoid bacterial contamination; rather, they expressed higher concerns about chemical risks than for microbial contaminants (14, 33). Several factors contribute to outbreaks of foodborne illness in the home. Most food eaten is prepared at home, thereby contributing to the likelihood for food handling mistakes to occur in this setting. In addition, most consumers consider the domestic environment a safe place (8, 61), thus underestimating the role of personal handling of products in contamination in the domestic environment. Moreover, home kitchens are multipurpose areas and are much more than just food preparation and storage places (58). Studies have shown that surfaces in the domestic environment are contaminated with pathogenic and non-pathogenic microorganisms and that, in some cases, kitchen locations are more contaminated with fecal coliforms than bathrooms (3, 9, 21, 53). Several consumer-based research studies have pointed out that after purchase of food,

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TABLE 1. *Ingredients, physical parameters, and shelf lives of the poultry-based meat preparations purchased in Northern Italy, as provided by the manufacturer*

| Product | Ingredients | Physical parameters of finished product | Shelf life (days) |
|--------------------------------------|---|---|-------------------|
| Meat patty formulation for burgers | Minced turkey meat (82%); water, wheat flour, ground rice, salt, sodium ascorbate, flavors (18%) | Wt, 104 ± 3 g; diam, 9.5 ± 0.5 cm; thickness, 1.3 ± 0.2 cm | 11 |
| Poultry sausage mixture for sausages | Minced chicken meat (50%); bacon (25%); water, salt, dextrose, saccharose, lactose, vegetable fibers, flavors, antioxidants E300 and E301, acidity corrector E331, spices, food color E120 (25%) | Wt, 75 g; diam, 2.3 cm | 10 |
| Kebabs | Chicken meat (54%); sausages (pork [35%]; turkey meat [28%]; water, chicken meat [8%]; salt, peas, carrots, dextrose, lactose, natural flavors, spices, antioxidants ascorbic acid, sodium ascorbate, sodium acetate) (34.5%); bacon (7%); peppers (4.5%) | Wt, 160 g; diam of chicken meat, 3.8 ± 0.6 cm; diam of sausages, 2.5 ± 0.3 cm | 9 |

improper transport, handling (cross-contamination), storage, and/or cooking frequently happens, thus allowing the survival, spread, and multiplication of microorganisms (1, 7, 31).

Among consumer food handling practices, time-temperature abuse has been reported as one of the most common contributory factors in salmonellosis outbreaks with strong evidence and in foodborne illness in general (15, 65). Several studies have been conducted on the temperature performance of domestic refrigerators, demonstrating that the majority of the refrigerators throughout the world are running at higher than recommended temperatures (0 to 5°C) and that the temperature can vary significantly according to the internal position measured (27). Moreover, Nauta et al. (45) hypothesized a geographical distribution of refrigerator temperatures, with the Northern countries usually recording lower mean refrigerator temperatures than the Southern countries. Studies conducted in the United Kingdom, Ireland, Greece, and New Zealand (18, 25, 26, 32, 35) showed that from 55 to 64% of refrigerators operate at a temperature of >5°C, and this goes up to 80% in the case of domestic refrigerators in France and Italy (10, 37). In addition, concerning storage temperature, from 44 to 75% of European consumers are not aware of the temperature of their domestic refrigerator (17, 36, 42).

Most consumers were not familiar with the expression “cold chain,” and only 10 to 20% of consumers bought perishable foods (like raw meat) at the end of shopping in order to reduce the potential for temperature abuse during transport home (29, 49). In regard to food transport, the majority of consumers used the car trunk to transport food items purchased at stores (19, 34), and transport took from 25 min (29) to more than 2 h (19, 30, 32). An insulated bag to maintain temperature while carrying meat or perishable food home was used by only 15 to 18% of consumers (19, 29), with the rate dropping to 5% in the case of Turkish consumers (30).

Freezing is a common type of storage adopted by 50 to 60% of European consumers in order to extend meat storage life (19, 29, 43, 56). Several of these studies reported that 46 to 60% of consumers preferred to thaw food on the kitchen countertop at room temperature (23 to 25°C), while only 26

to 30% thawed food in the refrigerator (2, 38) (personal communication).

The purpose of the present study was to reproduce consumers' storage and thawing practices in an experimental kitchen in order to record the effects of different refrigeration temperatures and thawing methods on the presence and numbers of *Salmonella* bacteria in naturally (by preference) or artificially contaminated poultry-based meat preparations (burgers, sausages, and ready-to-cook kebabs) commonly found in retail stores in Italy.

MATERIALS AND METHODS

Selection of poultry-based meat preparations and microbiological analyses performed on the day of delivery. Three poultry-based meat preparations were studied: burgers, sausages, and ready-to-cook kebabs. The ingredients, physical parameters, and shelf lives of the final products, as provided by the manufacturers, are shown (Table 1). The main ingredient was poultry meat (chicken or turkey); however, with the exception of the burgers, the products also contained pork or bacon. The products were obtained from local poultry meat industries in Northern Italy, which delivered the commercial poultry meat patty formulation (for burgers), commercial sausage mixture (for sausages), and ready-to-cook kebabs to the laboratory, at refrigeration temperature (4°C), within a maximum of 2 days from the time of production.

On the delivery day, for rapid detection of *Salmonella* bacteria, the poultry-based meat preparations were analyzed by real-time PCR using a commercial kit validated by the manufacturer (AES Chemunex, Biomerieux Company, Combourg, France) to comply with ISO 16140. A sensitivity of 98.11% (confidence interval, 89.92 to 99.95%) and specificity of 95.46% (confidence interval, 93.82 to 96.77%) were calculated for this PCR assay (39). Moreover, detection and quantification of *Salmonella* bacteria were also performed according to, respectively, the ISO 6579:2002/Amd 1:2007 (23) classical detection method for 25-g amounts of meat and the ISO/TS 6579-2:2012 (24) miniaturized most-probable-number (MPN) method, starting from the 1/10 primary suspension of 25 g of meat prepared for detection. Estimated numbers from the miniaturized MPN method were expressed as MPN/g; the lower limit of quantification was 1.3 MPN/g, and the upper limit of quantification was 710 MPN/g. Finally, all *Salmonella* bacteria detected were serotyped (White-Kauffmann-Le Minor scheme) by slide agglutination with O and H antigen-specific sera (Staten Serum Institute, Copenhagen, Denmark).

Before potential artificial contamination with *Salmonella enterica* serovar Typhimurium, 16 samples of the commercial poultry meat patty formulation were analyzed before preparing burgers, 18 commercial sausage mixture samples were analyzed before preparing sausages, and 36 kebabs were analyzed before experimental trials.

Storage and freezing-thawing tests were conducted on naturally contaminated products (positive PCR result) and artificially contaminated products (negative PCR result).

Artificial contamination procedure. A strain of *Salmonella* Typhimurium DT104 (2324/5 2010Salm strain, culture collection at the Italian *Salmonella* Reference Laboratory), isolated from minced poultry meat and kept in a cryobank, was spread on nutrient agar and incubated at 37°C for 18 h. Afterwards, the strain was suspended in buffered peptone water in order to obtain a suspension with an optical density at 600 nm of 1. Next, this suspension was serially diluted in buffered peptone water, and appropriate dilutions were used to obtain three residual contamination levels in the food products: ca. 10, ca. 100, and ca. 1,000 CFU/g.

In storage tests, only the 10 CFU/g level of artificial contamination was tested on poultry-based meat preparations, as this is considered a plausible level of contamination of poultry-based meat preparations available on the market, in light of both data in the literature (60) and tests performed on naturally contaminated products at the National Reference Laboratory for *Salmonella* in Italy (54). For the freezing-thawing tests, in order to be able to quantify any potential reduction of *Salmonella* bacteria due to the freezing-thawing practice, in addition to the level of contamination of 10 CFU/g, an additional two levels of contamination were tested, namely 100 and 1,000 CFU/g, considered the intermediate and worst-case scenario, respectively, in which *Salmonella* bacteria after accidental contamination (at low levels of <1 to 10 CFU/g) had already multiplied due to prior storage under temperature abuse.

According to the kind of test and, consequently, the number of samples needed, for burgers at each inoculum level, 7 to 12 ml of the appropriate dilution was added to 1,000 to 1,800 g of commercial poultry meat patty formulation and mixed thoroughly for five minutes in a planetary mixer (KitchenAid professional, model KMP05 PRO) with a flat aluminum beater, and portions pressed into a petri dish in order to obtain final burger weights and dimensions (Table 1). For sausages at each inoculum level, 6 to 12 ml of the appropriate dilution was added to 900 to 1,800 g of commercial sausage mixture and mixed thoroughly as described above, and the mixture stuffed into a bovine natural collagen gut casing using a commercial sausage filler, provided by a local sausage producer. Sausages 75 g in weight, 2.3 cm in diameter, and 15 cm in length were prepared. After each use, the sausage filler was sterilized (121°C for 30 min). Ready-to-cook kebabs were contaminated with *Salmonella* Typhimurium using a spray, which from previous tests (54) had proven to be the most efficient and effective contamination method. Each kebab was contaminated with 1.0 ml of the appropriate dilution for the level of contamination desired.

After artificial contamination, for each inoculum level, three replicates were analyzed to verify the presence and estimated numbers of *Salmonella* Typhimurium. Once artificially inoculated, burgers and sausages were packed in groups of three in stomacher bags, while kebabs were packed in groups of three in modified atmosphere (15% oxygen, 60% carbon dioxide, and 10% nitrogen) using a bell-shaped vacuum packer (Orved VM16).

Storage and freezing-thawing tests. Naturally or artificially contaminated poultry-based meat preparations were stored at three different temperatures (4, 8, or 12°C [$\pm 1^\circ\text{C}$]) during the shelf life period. The temperatures were selected in order to study the behavior of *Salmonella* in the food when it was stored according to label instructions (keep at 0 to 4°C) and at two different refrigeration temperatures, namely, 8 and 12°C, which are the temperatures recorded in 33% and 3% of domestic refrigerators, respectively, in a study performed in Italy (10).

For each type of contaminated product, three replicate samples were taken at two or three time points for detection and enumeration of *Salmonella* and were analyzed as described above. Burgers were analyzed after 4, 8, and 11 days of storage, while sausages, due to logistic difficulties in sending the commercial sausage mixture to the laboratory on a day as close as possible to the time of production, were analyzed after 7 and 10 days of storage. Kebabs were analyzed after 4, 7, and 9 days of storage: for each sample, portions from all the items on a kebab skewer were collected and homogenized in order to start the analyses.

Moreover, in keeping with the goal of simulating storage habits and behaviors that are likely to be adopted by consumers, poultry-based meat preparations were kept, after artificial contamination, at 25°C for 2 h (temperature abuse), in order to simulate a plausible scenario of not storing food in the refrigerator for a short time after purchase, as reported in the literature (32). After temperature abuse, products were stored in domestic refrigerators at one of three different temperatures, as previously described (storage tests). The core temperature of the products before and after temperature abuse was measured using a thermocouple (P200 Profi-Digital Thermometer, TFA, Wertheim, Germany) calibrated between 0°C (melting ice) and 100°C (boiling water) prior to use. For kebabs, the thermocouple was inserted into the center of one poultry meat piece, avoiding the wooden skewer.

Finally, freezing-thawing tests were carried out on artificially contaminated burgers, sausages, and kebabs, which were frozen at -22°C for 15 days and subsequently thawed overnight in disposable plastic containers at room temperature on the kitchen countertop (23°C) or in a domestic refrigerator (8°C). For each type of thawing method and level of contamination, three replicates were analyzed to detect and estimate the numbers of *Salmonella* Typhimurium bacteria.

Data analysis. In order to perform statistical tests, for each storage and thawing temperature, the results of the quantitative analyses performed on samples were analyzed together, irrespective of the time point and the level of artificial contamination. All analyses were performed with SPSS Statistics version 22 at a significance level of 95% ($P = 0.05$). The Kruskal-Wallis test was used for nonparametric statistical analysis of the *Salmonella* numbers in the different meat products at different temperatures. The Bonferroni correction was applied to control the familywise error rate at 5% for all multiple pairwise comparisons.

RESULTS

Storage tests: detection and quantification of *Salmonella* bacteria. Analysis of meat samples upon arrival in the laboratory provided the following results: 6 of 16 commercial poultry meat patty formulation samples contained *Salmonella* bacteria. Of the six positive samples, four provided enumerable results ranging from 1.3 to 3.8 MPN/g. *Salmonella* bacteria were detected in 8 of 18 sausage mixture samples at numbers below 1.3 MPN/g (except that one sample had 1.6 MPN/g), and finally, 1 of 36 kebabs was

found positive for *Salmonella* bacteria, leading to the decision to carry out storage tests only on artificially contaminated kebabs. The *Salmonella* isolates were serotyped: the poultry meat patty formulation contained mainly *S. enterica* serovar Newport (four of six isolates), as well as *Salmonella* Typhimurium and *S. enterica* serovar Brandenburg, while the sausage mixture and the kebabs contained *S. enterica* serovar Montevideo (all eight isolates) and the monophasic variant of *Salmonella* Typhimurium, respectively.

The results of the detection and estimation of numbers of *Salmonella* bacteria in naturally or artificially contaminated poultry-based meat preparations before (day zero) and after the storage tests are reported in Table 2.

The temperature abuse treatment at 25°C for 2 h did not affect the presence or numbers of *Salmonella* in naturally contaminated burgers and sausages. In fact, *Salmonella* bacteria were detected in 10 of 13 samples and 9 of 12 samples analyzed before and after temperature abuse, respectively, with numbers below or at the lower limit of detection of the MPN method. In artificially contaminated kebabs, *Salmonella* Typhimurium was always detected in the samples analyzed, with numbers ranging from 5 to 13 MPN/g before temperature abuse and from 3 to 8 MPN/g after temperature abuse. The recorded core temperatures before the 2 h of temperature abuse were, respectively, 3, 5, and 6°C for burgers, sausages, and kebabs, while the core temperatures after temperature abuse were 19, 17, and 18°C, respectively.

As expected, the storage temperature of 4°C did not facilitate an increase in the presence or estimated numbers of *Salmonella* bacteria in either naturally or artificially contaminated poultry-based meat preparations throughout the extent of the shelf life. In naturally contaminated samples (burgers and sausages), *Salmonella* bacteria were detected in 20 of 30 samples during the shelf life period established by the manufacturer, with levels of contamination at or below the lower limit of the MPN method. In the case of artificially contaminated samples (burgers, sausages, and kebabs), *Salmonella* Typhimurium bacteria were always detected in numbers comparable to those of the initial artificial inoculation at day zero.

The storage of products at 8°C led to different results for naturally and artificially contaminated samples. In fact, with regard to naturally contaminated burgers and sausages with or without temperature abuse before storage, *Salmonella* bacteria were detected in 18 of 26 samples at day zero in numbers at or below the lower detection limit of the MPN method. During the storage period (11 days for burgers and 10 days for sausages), *Salmonella* bacteria were detected in 14 of 30 samples in numbers at or below the MPN detection limit. In artificially contaminated samples, namely, burgers, sausages, and kebabs, *Salmonella* Typhimurium bacteria were always detected and estimated MPNs were obtained in 19 of 24 samples analyzed during storage with levels comparable to the numbers initially present after artificial contamination. Thus, at 8°C, most of the time, no substantial growth of *Salmonella* Typhimurium was noted, although in one of three sausages analyzed on day 7, a count of 380 MPN/g was estimated. In the case of kebabs stored at 8°C,

four of six samples analyzed at the end of the storage contained estimated numbers of *Salmonella* Typhimurium bacteria between 110 and 240 MPN/g.

The higher storage temperature of 12°C led to different results according to the type of meat preparation and type of contamination (natural versus artificial). With regard to naturally contaminated burgers (with or without temperature abuse before storage), *Salmonella* bacteria were detected in 12 of 16 samples before storage (with estimated numbers always at or below the lower MPN detection limit) and in 9 of 18 samples during the storage period (11 days). Of the nine positive samples, four contained estimated *Salmonella* numbers between 1.6 and 3.8 MPN/g. At this storage temperature, no growth of *Salmonella* occurred in naturally contaminated sausages (with or without temperature abuse before storage); in fact, *Salmonella* bacteria were detected in 9 of 13 sausage samples initially, in numbers at or below 1.3 MPN/g (the lower detection limit), and in 6 of 12 samples during the storage period (10 days) with numbers at or below the lower limit of the MPN method. However, in the case of sausages artificially contaminated with *Salmonella* Typhimurium (ca. 10 CFU/g), one of three samples contained 96 MPN/g at the end of the shelf life. Finally, in artificially contaminated kebabs (with or without temperature abuse at 25°C), statistically significant ($P < 0.05$) growth was recorded. In fact, the initial numbers of *Salmonella* Typhimurium bacteria (ca. 10 MPN/g) reached 710 MPN/g after 4 days of storage in two of six samples and exceeded the upper limit of the MPN method (710 MPN/g) at the end of the shelf life (days 7 and 9) for all 12 kebab samples.

Freezing-thawing tests: detection and quantification of *Salmonella* bacteria. In Table 3, the results of freezing-thawing tests on the detection and levels of *Salmonella* bacteria in artificially contaminated samples of burgers, sausages, and kebabs are reported. Some differences were noted in *Salmonella* Typhimurium numbers after freezing-thawing according to the kind of poultry-based meat preparation and the thawing temperature.

In particular, in the kebabs, overnight thawing at room temperature (23°C) caused a significant ($P < 0.05$) increase of *Salmonella* Typhimurium bacteria, with numbers exceeding the upper limit of the MPN method (>710 MPN/g) for all three levels of contamination tested. The thawing of the same kind of food in the refrigerator did not change the numbers of *Salmonella* Typhimurium for all three levels of contamination tested.

In the case of sausages, no *Salmonella* growth was observed after thawing at ambient temperature, as *Salmonella* numbers were maintained at the initial artificial inoculation level, while overnight thawing at refrigerator temperature (8°C) led to a significant ($P < 0.05$) decrease of *Salmonella* Typhimurium numbers.

Finally, for the burgers, lower numbers of *Salmonella* Typhimurium were recorded after thawing at both temperatures, but the difference was not statistically significant.

TABLE 2. Detection and numbers of *Salmonella* bacteria during storage tests, with or without temperature abuse, performed on naturally or artificially contaminated burgers, sausages, and kebabs

| Meat preparation | Day 0 ^a | | Temp (°C) | Day 4 | | Day 8 | | Day 11 | | |
|----------------------------|-------------------------------|--------------------------|--------------|-------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
| | Det. ^b | MPN/g ^c | | Det. | MPN/g | Det. | MPN/g | Det. | MPN/g | |
| Burgers | | | | | | | | | | |
| NC ^d | 7/8 | 1/8 (1.6) | 4 | 3/3 | 1/3 (1.3) | 3/3 | 0/3 | 3/3 | 0/3 | |
| | | | | 8 | 0/3 | 0/3 | 1/3 | 0/3 | 1/3 | 0/3 |
| | | | | 12 | 3/3 | 0/3 | 0/3 | 1/3 (3.8) | 3/3 | 0/3 |
| NC | Before temp abuse (2 h, 25°C) | | 4 | 1/3 | 0/3 | 2/3 | 0/3 | 2/3 | 0/3 | |
| | After temp abuse | | | 8 | 0/3 | 0/3 | 1/3 | 0/3 | 3/3 | 0/3 |
| | 6/9 | 0/9 | | 12 | 1/3 | 1/3 (3.2) | 1/3 | 1/3 (1.6) | 1/3 | 1/3 (1.6) |
| AC (10 CFU/g) ^e | 4/4 | 4/4 (8.1, 8.1, 8.1, 21) | 4 | 3/3 | 3/3 (3.2, 6.1, 12) | 3/3 | 3/3 (8.1, 8.1, 8.9) | 3/3 | 3/3 (1.6, 13, 41) | |
| | | | | 8 | 3/3 | 3/3 (1.6, 8.1, 19) | 3/3 | 3/3 (8.9, 13, 19) | 3/3 | 3/3 (8.9, 13, 19) |
| | | | | | | | | | | |
| Sausages | | | | | | | | | | |
| NC | 1/5 | 0/5 | 4 | 1/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | |
| | | | | 8 | 0/3 | 0/3 | 2/3 | 0/3 | 2/3 | 0/3 |
| | | | | 12 | 0/3 | 0/3 | 1/3 | 0/3 | 1/3 | 0/3 |
| NC | Before temp abuse (2 h, 25°C) | | 4 | 3/3 | 1/3 (1.6) | 2/3 | 0/3 | 2/3 | 0/3 | |
| | After temp abuse | | | 8 | 3/3 | 1/3 (6.1) | 3/3 | 1/3 (1.6) | 3/3 | 1/3 (1.6) |
| | 3/3 | 1/3 (1.6) | | 12 | 3/3 | 1/3 (1.6) | 2/3 | 0/3 | 2/3 | 0/3 |
| AC (10 CFU/g) | 3/3 | 3/3 (5.9, 18, 19) | 4 | 3/3 | 3/3 (1.6, 4.9, 8.5) | 3/3 | 3/3 (6.1, 12, 14) | 3/3 | 3/3 (6.1, 12, 14) | |
| | | | | 8 | 3/3 | 3/3 (5.9, 66, 380) | 3/3 | 3/3 (8.5, 18, 19) | 3/3 | 3/3 (8.5, 18, 19) |
| | | | | 12 | 3/3 | 3/3 (12, 31, 43) | 3/3 | 3/3 (12, 26, 96) | 3/3 | 3/3 (12, 26, 96) |
| Kebabs | | | | | | | | | | |
| AC (10 CFU/g) | 3/3 | 3/3 (1.6, 8.1, 13) | 4 | 3/3 | 3/3 (8.1, 31, 41) | 3/3 | 3/3 (1.4, 8.1, 13) | 2/3 | 3/3 (8.9, 12, 12) | |
| | | | | 8 | 3/3 | 3/3 (18, 21, 41) | 3/3 | 3/3 (11, 110, 240) | 2/3 | 3/3 (61, 170, 220) |
| | | | | 12 | 3/3 | 3/3 (21, 61, 710) | 3/3 | 3/3 (>710) | 3/3 | 3/3 (>710) |
| AC (10 CFU/g) | Before temp abuse (2 h, 25°C) | | 4 | 3/3 | 2/3 (3.2, 13) | 3/3 | 3/3 (3.1, 3.8, 13) | 3/3 | 3/3 (1.4, 1.4, 8.5) | |
| | After temp abuse | | | 8 | 3/3 | 2/3 (6.1, 8.1) | 3/3 | 3/3 (5.9, 6.1, 13) | 3/3 | 3/3 (1.4, 3.8, 6.1) |
| | 4/4 | 4/4 (3.2, 5.9, 8.1, 8.1) | | 12 | 3/3 | 3/3 (8.5, 110, 710) | 3/3 | 3/3 (2.8, 110, 710) | 3/3 | 3/3 (4.1, 20, 31) |

^a Day zero is the day of delivery or immediately after artificial contamination.

^b Det., detection per 25 g according to ISO 6579:2002/Amd 1:2007 (23). Number of positive samples/number of analyzed samples.

^c MPN/g, estimated number of surviving *Salmonella* bacteria according to ISO/TS 6579-2:2012 (24). Number of samples with a quantity above 1.3 MPN/g/number of analyzed samples.

^d NC, naturally contaminated.

^e AC, artificially contaminated.

DISCUSSION

Domestic kitchens and time-temperature abuse during domestic storage have been identified as the main setting and contributory factors involved in foodborne outbreaks (20, 62). Moreover, in the framework of “home food safety,” intended to describe the total sum of measures used to prevent contamination with pathogens, temperature abuse practices during transport of food and due to thawing methods can occur, as shown by consumer-based studies (5, 63). However, how such time-temperature fluctuating conditions affect the behavior of *Salmonella* bacteria, the preeminent causative

agent of foodborne outbreaks in the European Union, has been investigated only rarely (13, 22, 40, 59), while many studies have focused mainly on *Listeria monocytogenes*, due to its well known psychrotrophic nature. The present study, therefore, provides some data on the behavior of *Salmonella* bacteria in three different types of poultry-meat preparations (burgers, sausages, and kebabs) that were naturally or artificially contaminated with *Salmonella* and submitted to time-temperature abuse both during refrigerated storage (and also prior to storage, simulating shop-to-home transport) and during freezing-thawing.

TABLE 3. Detection and numbers of *Salmonella Typhimurium* bacteria on artificially contaminated burgers, sausages, and kebabs subjected to freezing-thawing tests

| Contamination conditions | Burgers | | | Sausages | | | Kebabs | | |
|--------------------------|------------------------|-----------------------|-----------|-----------------------|-----------|---------------------|-----------|---------------------------|--|
| | Detection ^a | MPN/g ^b | Detection | MPN/g | Detection | MPN/g | Detection | MPN/g | |
| 10 CFU/g | | | | | | | | | |
| After inoculation | 4/4 | 4/4 (3.8, 4.9, 8, 21) | 3/3 | 3/3 (3.8, 6.1, 8.1) | 3/3 | 3/3 (13, 21, 21) | 4/4 | 4/4 (6.1, 13, 45, 110) | |
| Thawing at 23°C | 2/3 | 2/3 (1.3, 3.8) | 3/3 | 3/3 (3.8, 13, 14) | 3/3 | | 3/3 | 3/3 (>710) | |
| Thawing at 8°C | 3/3 | 2/3 (1.4, 1.6) | | | 3/3 | 2/3 (1.6, 6.1) | 3/3 | 3/3 (3.2, 7.4, 17) | |
| 100 CFU/g | | | | | | | | | |
| After inoculation | 4/4 | 3/4 (4.1, 110, >710) | 3/3 | 3/3 (110, 170, 240) | 3/3 | 3/3 (380, 380, 710) | 4/4 | 4/4 (66, 110, 170, 380) | |
| Thawing at 23°C | 2/3 | 2/3 (8.8, 8.9) | 3/3 | 3/3 (66, 67, 240) | 3/3 | | 3/3 | 3/3 (>710) | |
| Thawing at 8°C | 3/3 | 3/3 (3.8, 8.9, 21) | | | 3/3 | 3/3 (12, 19, 21) | 3/3 | 3/3 (66, 96, 380) | |
| 1,000 CFU/g | | | | | | | | | |
| After inoculation | 3/4 | 3/4 (21, >710, >710) | 3/3 | 3/3 (3.2, >710, >710) | 3/3 | 3/3 (>710) | 4/4 | 4/4 (380, 710, 710, >710) | |
| Thawing at 23°C | 3/3 | 3/3 (6.7, 45, 380) | 3/3 | 3/3 (710, 710, >710) | 3/3 | | 3/3 | 3/3 (>710) | |
| Thawing at 8°C | 3/3 | 3/3 (3.2, 6.9, 11) | | | 3/3 | 3/3 (15, 45, 56) | 3/3 | 3/3 (710, >710, >710) | |

^a Detection per 25 g according to ISO 6579:2002/Amd 1:2007 (23). Number of positive samples/number of analyzed samples.

^b MPN/g, estimated number of surviving *Salmonella* bacteria according to ISO/TS 6579-2:2012 (24). Number of samples with a quantity above 1.3 MPN/g/number of analyzed samples.

With regard to the temperature abuse prior to refrigerated storage, the study of Kim et al. (34) highlighted that refrigerated foods (e.g., eggs, milk, and fresh meat) left in the car trunk exposed to sunlight reached 20°C within 40 min and 30°C within 90 to 110 min, thus reaching the temperature danger zone (5 to 60°C) for foodborne pathogens to grow. For this reason, the refrigeration of perishable foods within 2 h is recommended, and when the outdoor temperature reaches 32.2°C, the time between purchase and refrigerated storage should be reduced to 1 h (64). In the present study, exposure of the poultry-based meat preparations to 25°C for 2 h before refrigerated storage did not have any effect on the presence or estimated numbers of *Salmonella* bacteria in either the naturally or the artificially contaminated poultry-based meat preparations. This was in spite of the average internal temperatures of the products recorded after this temperature abuse ranging from 17 to 20°C and, thus, being above the minimum growth temperature of *Salmonella* bacteria. It is likely that the 2 h of temperature abuse was still within the microorganism's lag phase, and therefore, growth of *Salmonella* was not observed. This lends support to the *Salmonella*-related safety of the 2-h rule, which is commonly used as a maximum guideline time for the nonrefrigerated holding of perishable products.

Our observations on the stability of the presence and estimated numbers of *Salmonella* bacteria during refrigerated storage at 4°C are supported by previous studies on *Salmonella* survival in a variety of artificially contaminated (>3 log units) raw chicken products during refrigerated storage (4, 6, 47, 50, 52). In these studies, *Salmonella* could survive but was not able to proliferate at 4°C during storage periods ranging from 8 to 21 days. In fact, food safety authorities (e.g., the Food Standards Agency and the U.S. Department of Agriculture) of different countries recommend that refrigerated foods be stored between 4 and 5°C as an intrinsic part of safe food handling, in order to inhibit or prevent the growth of spoilage and pathogenic organisms, such as *Salmonella*.

Storage at 8°C, for most of the meat products sampled, did not support an increase in the presence and estimated numbers of *Salmonella* bacteria, although in a few artificially contaminated sausages and kebabs, the estimated numbers of *Salmonella Typhimurium* bacteria were on the order of 2 log units. The latter findings are in agreement with studies conducted on artificially contaminated (>3 log units) chicken meat samples (chicken, breast, and thighs) stored at 8°C, in which significant ($P < 0.05$) increases of *Salmonella* numbers within 9 days of storage were recorded (6, 52, 66).

Storage at 12°C facilitated a significant ($P < 0.05$) increase of *Salmonella Typhimurium* bacteria in artificially contaminated kebabs (10 CFU/g). In fact, *Salmonella* numbers exceeding the upper limit of the MPN method (>710 MPN/g) were found in seven of nine kebabs analyzed during the shelf life period. Studies investigating the behavior of *Salmonella* on artificially inoculated chicken meat stored at 12°C observed growth of *Salmonella* from 10 CFU/g to 2,900 CFU/g within the 9-day shelf life (6, 47, 48, 66), thus confirming that improper refrigeration tempera-

tures can have a substantial effect on the growth of this pathogen.

With regard to freezing-thawing, other studies investigating the behavior of *Salmonella* bacteria during frozen storage concluded that *Salmonella* could survive at frozen storage temperatures (12, 52). However, how thawing practices could affect the numbers of *Salmonella* in meat products has rarely been investigated. The study of Lianou and Koutsoumanis (40) observed no significant changes in *Salmonella* Enteritidis counts on artificially contaminated ground beef samples during abusive thawing (25°C for 12 h), while according to Manios and Skandamis (41), thawing of beef patty samples on the kitchen counter (20°C for 12 h) resulted in significant increases in *Salmonella* populations. Statistical tests performed on the thawing data collected in this study led in some cases to nonsignificant results. This is mainly related to the fact that in order to have a sufficient number of samples to be analyzed, for each kind of food and thawing method, the estimated numbers of *Salmonella* bacteria from the three different levels of contamination were analyzed together. However, the present study highlighted that the type of thawing method and the kind of food affect the numbers of *Salmonella*. In fact, thawing overnight in the refrigerator (8°C) led to either a moderate reduction or no change in *Salmonella* Typhimurium numbers in burgers, sausages, and kebabs. Thawing overnight on the kitchen countertop (23°C) caused significant increases in *Salmonella* Typhimurium numbers in kebabs, but occasionally, the numbers remained stable or were even reduced in sausages and burgers, respectively. This phenomenon could be explained by possible different time-temperature profiles, as described by Manios and Skandamis (41), of different food matrices. Consequently, the recommendation to defrost poultry meat at a temperature between 5°C and 7°C, i.e., in a refrigerator, is still pertinent in order to prevent the growth of microorganisms like *Salmonella* (11), as was observed (although not consistently) in the present study.

Overall, the results of the present study highlighted that domestic and consumer-related storage and thawing practices could affect poultry meat safety in regard to *Salmonella*. Time-temperature abuse allowed a substantial increase of *Salmonella* numbers in poultry-based meat preparations, although the actual observed behavior of *Salmonella* (no change in presence or numbers, either reduction or growth) is dependent upon several factors, such as the exact extent of temperature abuse (with 12°C being more supportive for growth than 8°C), the type of strain (with the artificially inoculated strain facilitating growth and notably larger increases in *Salmonella* numbers upon temperature abuse than natural strains) and the type of meat preparation (with kebabs being more supportive of growth than sausages and burgers).

The latter result could be due to the fact that, even though the kebabs were packed in a modified atmosphere, while burgers and sausages were not, they were composed of pieces of whole meat tissue. Thus, while burgers and sausages contained added salt and spices, likely with an inhibitory effect on bacterial growth (57), the structure and composition of kebabs in the packaging may have allowed

the presence of more exudates, which can provide nutrients to bacteria. In addition, we were unable to detect any naturally occurring *Salmonella* bacteria among the kebabs, and so these were studied only with artificial *Salmonella* contamination. In contrast to fully viable bacteria used for artificial contamination, bacterial cells in naturally contaminated foods are frequently impaired by sublethal injury as a result of having been exposed to adverse conditions during food processing (28). Therefore, retarded growth due to a longer lag phase might have occurred in the present study.

Although the results of the present study, using poultry-based meat preparations that were both naturally and artificially contaminated with *Salmonella*, show that temperature abuse (prior to storage, during refrigerated storage, or during freezing-thawing) did not consistently, for all samples, lead to a substantial increase in the presence or estimated numbers of *Salmonella* bacteria in the products under consideration, there was occasional substantial growth of *Salmonella* in artificially contaminated samples. Therefore, efforts to disseminate guidelines for consumers on correct storage and handling of food need to be continued. This is of particular importance given the widespread lack of consumer knowledge of safe food handling practices in the kitchen. Studies have reported that large proportions of consumers (up to 93%) do not know the recommended refrigerator operating temperature range and do not have a thermometer with which to measure it (16, 46). Part of this lack of knowledge may stem from the fact that most consumers do not consider themselves responsible for food safety to the same degree as professional food handlers (29, 49). According to Rosati and Saba (55), the majority of Italian consumers identify the food industry and public institutions as bearing the main responsibility for assuring food safety, while considering themselves as having the least responsibility. On-going efforts taken by food safety authorities and organizations such as the International Scientific Forum on Home Hygiene remain necessary to set up effective educational campaigns addressing specific topics of consumer food handling-related behaviors.

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