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Thermal Inactivation of *Salmonella* on Sesame-topped Bread during Baking Using High and Low Oven Humidity

ABSTRACT

Unpasteurized ingredients, such as contaminated sesame seeds or flour, may introduce *Salmonella* into bakery products. Therefore, the use of a validated baking process is critical to eliminate this pathogen. This study compared the effect of oven relative humidity (RH) on the reduction of *Salmonella* and yeast during baking of inoculated bread dough topped with inoculated sesame seeds. *Salmonella* was injected into individual portions of bun dough to yield populations of 7.0–8.0 log CFU/g; dough was proofed and then topped with dry, inoculated sesame seeds (6.0–7.0 log CFU *Salmonella*/g seed). The seeded buns and seeds alone were baked in dry (average ~3% RH) or moist (average 20% RH after wet bulb spike) oven conditions for 7 and 9 min, respectively, to match a "bun-color standard," as suggested by a commercial bakery. After complete baking, *Salmonella* and yeast populations in the bun had decreased > 5-log for both methods. In contrast, differences in oven RH affected inactivation on the surface seeds, with reductions of 3.6 and > 6.0 log *Salmonella* at the end of dry and moist baking, respectively. This study demonstrates that maintaining an average 20% relative humidity during baking enhances the lethality on the product surface, compared to ≤ 3% RH, while also maintaining quality.

INTRODUCTION

Foods such as meat, poultry, eggs, fresh produce, spices, nuts, and dairy products are the most common vehicles of foodborne salmonellosis worldwide, but flour-based products, including bakery items, have also been implicated in outbreaks *(8, 13, 18, 19, 27, 31)*. *Salmonella* may be introduced into bakery products through a wide range of raw agricultural commodities and unpasteurized ingredients, either low or high in water activity (a_w), such as raw flour, egg, dried dairy ingredients, spices, nuts or seeds *(19, 24, 35)*. Recently, several *Salmonella* outbreaks and recalls have been associated with sesame seeds and contaminated sesame

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seed-based food products *(6, 29, 32)*. Survey data reveal that 0.14% of U.S. wheat flour and 9.9% of imported sesame seed samples were Salmonella positive, but populations were not enumerated *(26, 30)*. Outbreak investigations suggest that the infective dose may be fewer than ten *Salmonella* cells per 100 g of food; hence the pathogen must be eliminated from ready-to-eat foods *(2, 10, 19)*.

Interactions between factors such as temperature, atmosphere and a_w play an important role in *Salmonella* survival and heat resistance *(22)*. Although *Salmonella* does not grow in bakery products, seeds or nuts because of the low a_w, the organism may survive for relatively long periods in the food *(4, 5)*. For example, *Salmonella enterica* serotype Infantis and *S.* Typhimurium were viable (1–2 log reduction) in dried pasta after 360 days of room temperature storage *(23)*. *S.* Enteritidis survived (< 2 log reduction) eight months of room temperature storage in air-sealed packed halva (a_w 0.18); which contains sesame seeds as a main ingredient *(15)*. Likewise, *Salmonella* is extremely resistant to inactivation by dry heat. Heating *Salmonella*-inoculated non-fat dried milk powder (4% moisture; low water-activity product) in an oven at 115.5°C for 15 min, using a dry heat treatment, resulted in only 0.8 log reduction *(21)*. Oil roasting of almonds at 127°C requires 1.6 and 2.0 min to achieve a 4 and 5-log reduction of *Salmonella*, respectively *(1)*.

A high final internal product temperature (e.g., 90–95°C) is typically required for functionality and quality of bakery products. Oven humidity has also been shown to affect the color, baking time, crust and crumb texture, and final product moisture *(33, 34)*. While it is presumed that standard baking procedures are sufficient to inactivate normal populations of *Salmonella* in bakery items, few validated processes have been published *(9, 16, 17)*. Thermal inactivation rate depends on a number of inter-related factors, such as the composition of the bakery product (pH, a_w, preservative), level of contamination, and time and temperature of the heating process *(24)*. A parameter that may affect the survival of *Salmonella*, but is less defined, is the humidity of the oven during baking, which directly affects evaporative cooling at the product surface. Evaporative cooling, influenced primarily by low oven moisture and air velocity during thermal treatments, has been associated with lower lethality, whereas wet bulb spikes can promote lethality *(11, 20)*. However, little information is available on the effect that this factor has on inactivation rates on contaminated bakery ingredients and toppings. Hence, the objective of this study was to compare the effect of oven relative humidity on the reduction of *Salmonella* during baking of inoculated buns topped with inoculated sesame seeds.

MATERIALS AND METHODS

Salmonella inoculum preparation

A six serovar mixture of *Salmonella enterica*, consisting of Enteritidis E8864 (cheesecake isolate), Enteritidis E40 (chicken ovary isolate), Enteritidis PT30 (raw almond isolate), Heidelberg S13 (human isolate), Typhimurium S9 (human isolate), and Typhimurium A1 (peanut butter isolate), was used in this study. Stocks of these strains were maintained on ceramic beads (CRYO/M; Copan Diagnostics Inc., Murrieta, CA) and stored at -80°C. For inoculum preparation, each individual strain was cultured in 10 ml of fresh tryptic soy broth (TSB; Becton, Dickinson and Company, Sparks, MD, USA) at 37°C for 20 to 24 h. The freshly grown culture (0.1 ml) was further transferred onto tryptic soy agar plates (TSA) and incubated at 37°C for 18–22 h to produce a lawn of bacterial cells. The cells were harvested using sterile cotton swabs and by suspending them in 9 ml of 0.1% peptone water (PW). Strains were mixed in equal concentrations to deliver approximately 8 log CFU/g of product. Populations of the individual strains and multi-strain mixture were verified by plating serial dilutions on xylose lysine desoxycholate agar (XLD; Becton, Dickinson and Company); each strain was also verified for purity by streaking on TSA. All plates were incubated at 37°C for 24 hours.

Inoculated sesame seed preparation

Salmonella-contaminated sesame seeds were prepared by pipetting 4 ml of the *Salmonella* inoculum dropwise into 200 g sesame seeds contained in a sterile glass beaker and intermittently stirring the mixture with an ethanol-sanitized spatula. The beaker was covered with sanitized aluminum foil and the product inside was hand-agitated for four to five minutes to further distribute the inoculum. The seeds were air dried overnight at room temperature (20 h; 22–23°C) in a biosafety cabinet by spreading in a thin layer over sterile aluminum foil. The viable *Salmonella* populations were determined by plating on XLD agar after drying. Water activity of the uninoculated seeds was 0.47 ± 0.02; after drying, the a_w was ≤ 0.45.

Baking temperature, time, and relative humidity

Baking was carried out in a Combitherm Oven (6.10ESI SK; Alto Shaam Inc., Menomonee Falls, WI) preheated to 177°C (350°F) for dry RH baking and 204°C (400°F) for moist RH baking experiments, per recommendations by a commercial bakery (ARYZTA). During moist RH baking process, relative humidity inside the oven was maintained at a target average of 20% after wet bulb spike by injecting steam (oven built-in to spray for 15 sec) at three different time points during baking (15 sec, 3 min, and 5 min), whereas no steam was added for dry RH baking. The timing of steam injection was determined by carrying out preliminary trials in order to maintain the target average humidity. The times of baking under both conditions were determined by preliminary trials conducted to obtain products comparable to those with a standard color ("golden brown") provided by the bakery. Using these parameters, baking was performed for 7 and 9 min, respectively, for dry and moist RH baking experiments.

Bread dough inoculation and baking

A commercial bakery shipped pre-formed, frozen bun dough to UW-Madison, where it was stored at -20°C until use within 4 weeks. The ingredient statement listed: enriched flour (bleached wheat flour, malted barley flour, niacin, reduced iron, thiamin mononitrate, riboflavin, folic acid, enzymes), water, high fructose corn syrup, yeast *(Saccharomyces cerevisiae)*, sugar, soybean oil and/or partially hydrogenated soybean oil; contains 2% or less of the following: salt, calcium sulfate, calcium carbonate, wheat gluten, ammonium sulfate, ammonium chloride, dough conditioners (sodium stearoyl lactylate, datem, ascorbic acid, azodicarbonamide, mono- and diglycerides, ethoxylated monoglycerides, monocalcium phosphate, enzymes, guar gum, calcium peroxide, soy flour), calcium propionate and sodium propionate (preservatives), soy lecithin. The Pans (American Pan Company, Urbana, OH) used for the baking of buns contained eight pockets for individual buns; each round pocket was 10.2 cm in diameter and 1.6 cm deep. Prior to the experiment, pans were cut into halves to ease pulling trays from the oven during baking. Each half contained four pockets. Two half-pans were used on each of the three shelves in the oven.

For each experiment, frozen dough buns (85 g) were placed in baking pans to thaw at room temperature (about 22°C) for ~ 30 min. Individual thawed buns were inoculated with the *Salmonella* mixture to yield approximately 7 to 8-log CFU per g by injecting 0.85 ml inoculum (1%) into eight different areas to evenly distribute the inoculum throughout the bun, including the approximate geo-center of the bun (presumably the region of slowest heating). A total of eighteen inoculated buns were equally distributed and placed into the six half-pans. An additional four uninoculated dough buns were also placed in the pans to monitor internal and surface temperatures and to allow for photographing surface color during and after baking.

Inoculated and uninoculated dough buns were proofed at 43°C (110°F) for 60 minutes in the Combitherm Oven in steam mode, with the blower set at half speed. The proofed buns were removed from the oven for approximately 15 min to top the buns with inoculated sesame seeds, as well as to allow time to preheat the oven for baking. Three buns (one from each shelf) were removed as 0-time, unseeded samples; the remaining buns were sprayed with water (~14 µL per bun) on the upper surface, and topped with inoculated sesame seeds (1.5 g/bun). After seeding, three buns were removed as 0-time seeded bun samples. For the moist baking experiment only, six aluminum dishes (7.9 cm diameter), each containing 1.5 g of inoculated sesame seeds (prepared as described in a previous paragraph) spread in a single seed layer, were also placed in the empty pan pockets (one palette in every half pan). The remaining seeded buns and seeds alone were then baked in the preheated oven using convection mode and blower set to full speed (horizontal air flow) for 7 min at 177°C air temperature for dry baking and 9 min at 204°C air temperature for moist baking.

The internal temperature for one bun per baking trial was monitored every 5 sec by inserting a thermocouple probe (type K) into the geometric center of the bun and using a temperature recorder (4-Channel Temperature Meter/ Datalogger; SDL200; EXTECH Instruments, Nashua, NH). A surface probe thermocouple (SA1XL-K-72; Omega Engineering Inc., Stamford, CT) was placed on the upper surface of a bun to monitor the surface temperature.

Dry bulb and wet bulb temperatures of the oven also were recorded to calculate the relative humidity during baking. A wet-bulb probe was made as previously described *(14)*. Briefly, a thermocouple probe was draped with "wet bulb sock" (part number 50041; ALKAR, Lodi, WI) and the end of the sock was submerged in water contained in a glass beaker. The "wet-bulb probe" was placed in the bottom of the right-hand side of the oven, opposite the oven fan to minimize excessive evaporation of water. Dry bulb temperature was measured by placing the probe next to the top shelf, approximately at the midpoint of the shelf.

Microbiological and physicochemical analysis of samples

Two sets of samples were assayed at each baking interval for dry baking, and three sets of samples were assayed for moist baking (triplicate samples of each). For one set, a representative 25 g portion (including seeds if applicable) from each dough/bun was removed for microbial enumeration. The entire bun was macerated (dough sample) or hand flattened and cut into ~ 5mm pieces (partial or fully baked bun) and a representative 25 g composite sample from each bun was homogenized with 50 ml chilled (4°C) 0.1% peptone water (PW) for 2 minutes using a paddle blender (Stomacher® 400 Circulator, Seward LTD., UK Worthing, West Sussex, UK). For a second set, the seeds were isolated from the surface of each individual bun and the entire sample, which included some residual bread, weighed and homogenized with two volumes of PW. For the moist baking treatment only, samples that contained inoculated seeds only (1.5 g contained in aluminum cups) were mixed with 2 volumes of PW (3.0 ml). Bread samples were assayed for *Salmonella*, yeasts, lactic acid bacteria, and total plate count, after inoculation, after proofing, after seeding, at the mid-point of baking (3.5 or 4.5 min) and at the end of baking (7.0 or 9.0 min). Seeds were assayed for *Salmonella* and total plate counts only after seed inoculation, after drying (20 hours at room temperature), and at the mid-point and end of baking.

Immediately after removal from the oven, bread was placed inside a Whirlpak® bag (2040 mL; Nasco, Fort Atkinson, WI) and immersed in ice-water bath to chill samples. Likewise, samples of inoculated seeds only (1.5 g; moist baking experiment only) contained in aluminum palette cups were placed over the ice-water bath to prevent further heating.

Serial dilutions (1:10) of inoculated samples were surface plated for recovery of *Salmonella* on XLD plates (Becton, Dickinson and Company) incubated at 37°C for 24 hours or of heat-injured *Salmonella* on XLD Thin Agar Layer Plates comprised of XLD agar covered with TSA *(12)* incubated at 37°C for 24 to 48 hours. Likewise, inoculated samples were plated for yeasts on Potato Dextrose agar (acidified to pH 3.5 with 10% tartaric acid and incubated at 25°C for 3–5 days; Becton, Dickinson and Company), lactic acid bacteria in APT agar with 0.002% bromocresol purple (incubated at 37°C for 48 h; Becton, Dickinson and Company), and total plate counts in PCA agar (incubated at 37°C for 48 h; Becton, Dickinson and Company). In addition, triplicate uninoculated dough buns and sesame seed samples were assayed for indigenous populations of yeast, lactic acid bacteria, and total plate counts as described above. Colonies on each medium type were examined microscopically to confirm as yeasts, molds, or bacteria.

Triplicate uninoculated dough samples were analyzed for their physicochemical composition. Moisture content was measured as weight loss on drying (AOAC 934.01 *(3)*; 95–100°C for 5 h; vacuum oven E191047, VWR International LLC, West Chester, PA). The pH was measured on a slurry obtained by homogenizing 10 g dough/bread with 90 mL deionized water (Orion Star A111 benchtop pH meter and Orion 8104 combination electrode; Thermo Fisher Scientific, Waltham, MA). Water activity was measured using an AquaLab TE4 water activity meter (Decagon Devices Inc., Pullman, WA) and salt was measured as % Cl- by potentiometric titration against 0.1N AgNO3 solution, using an automated titrator (DL22 Food and Beverage Analyzer; Mettler Toledo AG, Schwerzenbach, Switzerland). In addition, moisture and a_w were measured for outer crust and inner portions of bun at the end of baking, and for seeds before inoculation, after drying, and after baking.

Statistical analysis

This study compared the reduction of *Salmonella* populations in buns when baked at either dry or moist relative humidity (RH ≤ 3% and average 20%, respectively) in the oven. Two independent trials were conducted for each method. *Salmonella* reduction was examined in the composite bun with seed samples, as well as in the sesame seeds portion alone, taken off the bun. In moist RH baking, additional samples consisted of sesame seeds alone, baked along with other samples. Microbiological data were transformed into log values for analysis. When colonies were not detected by direct plating in the lowest dilution samples, the minimum detection limit (1.78 log CFU/g for composite samples and 1 log CFU/g for seeds alone samples) was used for data analysis. A repeated measure design was used for statistical analysis. Analysis of variance for the repeated measures was performed using the MIXED procedure of SAS (version 9.3; SAS Institute Inc., Cary, NC). Compound Symmetry was used as the covariance structure. Means were separated using Tukey's method at a 5% level of significance. Data are presented as average ± standard deviations of two trials.

RESULTS AND DISCUSSION

Proximate analysis

The moisture, salt, a_w and pH values of the dough are presented in *Table 1*. As expected, the moisture content and a_w decreased during baking and were significantly less ($P \leq 0.05$) in the crust than in the inner portions with both baking methods. Moisture decreased from an average of 40.8 ± 0.5% in the dough to 39.0 ± 0.8% in the inner portion for both baking methods, but to 33.0 ± 1.3 and 35.5 ± 1.5% moisture for the outer crust in the dry and moist RH baking methods, respectively. Likewise, the a_w decreased from an average of 0.957 ± 0.005 in the dough to 0.946 ± 0.003 in the inner portion for both baking methods, and to 0.927 ± 0.15 and 0.939 ± 0.010 for the outer crust in the dry and moist RH baking methods, respectively. In both baking methods, the moisture loss on baking reduced the weight of the baked product to approximately 75 ± 3 g from the initial weight 85 ± 4 g of a dough bun. Similarly, the moisture content and a_w of inoculated dry sesame seeds decreased from 3.3 ± 0.0% and 0.269 ± 0.002 to 1.0 ± 0.1% and 0.153 ± 0.021, respectively, after 9 min of moist RH baking.

Temperature profiles

The temperature profiles of bun and oven air (dry and wet bulb), as well as the calculated relative humidity (RH) during baking are presented in *Tables 2 and 3*. For ease of reading, the data are presented for every 30 sec, although the temperatures were recorded every 5 sec. The oven was preheated to 176.7 and 204.4°C (350 and 400°F) in dry and moist RH baking experiments, respectively. As reported previously *(33, 34)*, baking at moist RH required a longer time (9 vs. 7 min) to produce the desired bun color, even though the oven temperature was set higher. Opening the oven door to load the samples lowered the starting temperatures (dry bulb) to 125.6 and 110.6°C (258 and 231°F) in dry and moist baking experiments, respectively. In both cases, internal temperature of the bun increased from an average of 32°C at 0-time to an average of 76.5°C at 6 min, even though air temperatures for the two methods were different. Dry bulb temperatures for the moist RH treatments were more variable and consistently lower than for dry RH treatments and never exceeded 175°C, because of the injection of moisture into the chamber.

In dry RH baking, the internal temperature of dough increased from an average of 32.2 to 46.7°C (90°F to 116°F) in 3.5 min (halfway sampling point). A minimum temperature of 73.9°C (165°F) was reached at 6 min and continued to increase to an average of 89.4°C (193°F) during the remainder of the 7 min baking. Similarly, a temperature of 66.1°C (151°F) was reached in 4.5 min of moist RH baking,

Table 1. The physicochemical composition of bun dough and buns (crust and inner portions) fully baked at dry and moist oven relative-humidity (≤ 3 and average 20% RH).

	Bun dough	Bun baked at ≤ 3% RH		Bun baked at avg. 20% RH	
Parameter		Crust	Inner	Crust	Inner
Moisture (%w/w)	40.8 ± 0.5	33.0 ± 1.3	39.0 ± 0.6	35.5 ± 1.5	39.0 ± 0.9
Salt (NaCl, %w/w)	1.1 ± 0.0	Na*	Na	Na	Na
pH	5.16 ± 0.06	Na	Na	Na	Na
a_w	0.957 ± 0.005	0.927 ± 0.015	0.944 ± 0.003	0.939 ± 0.010	0.948 ± 0.002

Data presented as means ± standard deviations of three samples for each trials (total n = 12 for dough; n = 6 for moist and dry baking)
*Na, Not analyzed

Table 2. Temperature and relative humidity (RH) recorded during baking in oven at relative humidity 2.3 ± 0.9%.

Time (min)	Product temperature (°C)		Oven temperature (°C)		Oven
	Internal	Surface	Dry bulb	Wet bulb	RH (%)
0.0	32.2 ± 0.4	69.2 ± 12.5	125.6 ± 8.4	40.2 ± 8.2	2.3 ± 0.6
0.5	32.1 ± 0.0	105.2 ± 24.9	143.0 ± 3.9	47.3 ± 9.0	2.2 ± 0.9
1.0	32.4 ± 0.1	113.1 ± 18.7	153.3 ± 3.9	51.6 ± 10.7	2.2 ± 1.1
1.5	32.9 ± 0.1	116.3 ± 14.5	163.0 ± 3.7	55.5 ± 11.7	2.2 ± 1.2
2.0	34.2 ± 0.1	123.9 ± 10.5	170.9 ± 3.7	58.6 ± 12.2	2.1 ± 1.2
2.5	37.0 ± 0.4	130.9 ± 6.3	175.0 ± 1.9	61.3 ± 12.0	2.3 ± 1.4
3.0	40.8 ± 0.2	135.3 ± 7.8	170.6 ± 4.2	63.2 ± 12.0	2.9 ± 1.9
3.5	46.5 ± 1.6	138.1 ± 8.2	167.6 ± 2.4	64.8 ± 12.4	3.3 ± 2.1
4.0	52.1 ± 2.7	132.7 ± 4.5	162.3 ± 2.0	49.4 ± 6.4	1.5 ± 0.5
4.5	57.7 ± 3.8	146.0 ± 8.8	173.1 ± 1.8	54.0 ± 8.9	1.5 ± 0.7
5.0	63.5 ± 5.0	153.0 ± 11.4	177.6 ± 1.3	57.7 ± 8.9	1.7 ± 0.8
5.5	69.8 ± 5.5	152.3 ± 10.9	173.3 ± 1.6	60.2 ± 9.0	2.2 ± 1.1
6.0	76.4 ± 5.1	150.5 ± 10.0	170.1 ± 1.7	62.1 ± 9.0	2.6 ± 1.2
6.5	82.6 ± 3.3	151.8 ± 6.2	172.2 ± 4.9	63.8 ± 9.0	2.6 ± 0.9
7.0	89.4 ± 0.0	155.9 ± 9.0	176.1 ± 1.2	65.4 ± 8.3	2.6 ± 1.1

Data are expressed as mean ± standard deviation of the two replications.

Table 3. Temperature and relative humidity (RH) recorded during baking in oven at average relative-humidity 20%.

Time (min)	Product temperature (°C)		Oven temperature (°C)		Oven
	Internal	Surface	Dry	Wet	RH (%)
0.0	32.1 ± 8.1	95.3 ± 1.8	110.4 ± 16.2	51.9 ± 0.3	8.3 ± 4.5
0.5	33.1 ± 10.0	113.8 ± 3.4	129.7 ± 2.8	79.9 ± 0.9	16.5 ± 0.7
1.0	35.5 ± 13.6	114.2 ± 15.4	132.6 ± 3.0	94.3 ± 0.9	27.4 ± 1.4
1.5	35.6 ± 13.6	129.9 ± 11.2	142.7 ± 4.9	95.0 ± 0.7	21.3 ± 2.3
2.0	35.0 ± 12.3	137.6 ± 12.5	150.9 ± 5.4	95.3 ± 0.8	17.4 ± 1.9
2.5	36.1 ± 11.9	146.5 ± 17.8	158.7 ± 5.2	95.4 ± 0.9	14.3 ± 1.4
3.0	39.9 ± 12.1	153.1 ± 18.8	165.8 ± 5.0	95.5 ± 0.7	12.1 ± 1.1
3.5	46.0 ± 12.1	128.5 ± 18.4	146.1 ± 2.4	97.0 ± 0.3	20.9 ± 1.1
4.0	55.0 ± 9.5	129.6 ± 11.0	142.7 ± 3.1	98.0 ± 0.4	23.9 ± 1.7
4.5	66.0 ± 10.6	118.9 ± 3.0	127.5 ± 38.1	88.2 ± 14.3	28.9 ± 15.8
5.0	67.7 ± 7.4	112.5 ± 4.9	110.0 ± 15.0	55.5 ± 3.1	9.8 ± 3.3
5.5	72.5 ± 5.7	106.7 ± 11.5	120.2 ± 3.7	85.1 ± 3.2	28.1 ± 7.0
6.0	77.1 ± 4.8	117.4 ± 2.8	122.3 ± 2.5	94.0 ± 0.6	37.1 ± 2.1
6.5	81.1 ± 3.9	124.9 ± 1.7	130.3 ± 6.0	95.3 ± 0.0	30.8 ± 5.4
7.0	84.1 ± 1.2	133.4 ± 2.9	139.1 ± 5.3	95.5 ± 0.2	24.0 ± 3.4
7.5	88.2 ± 0.7	140.6 ± 4.7	147.9 ± 5.7	95.7 ± 0.2	19.1 ± 2.7
8.0	90.9 ± 0.2	147.5 ± 5.1	155.9 ± 5.7	95.9 ± 0.2	15.7 ± 2.1
8.5	95.2 ± 2.9	154.1 ± 5.9	163.3 ± 6.0	96.1 ± 0.2	13.2 ± 1.8
9.0	96.7 ± 2.9	159.0 ± 6.4	168.7 ± 6.1	96.3 ± 0.2	11.7 ± 1.5

Data are expressed as mean ± standard deviation of the two replications.

with a minimum of 73.9°C (165°F) at 6 min, and continued to increase to 96.7°C (206°F) at 9 min. The average internal and surface temperatures of the bun were 51.4 and 61.3°C and surface temperature 132.7 and 130.2°C in the dry and moist RH baking, respectively.

The difference between dough surface and oven dry bulb temperatures was consistently greater in dry RH baking; the average surface temperature of dough was 31.9°C less in dry RH baking but only 10.6°C less in moist RH. The average dry and wet bulb temperatures of the oven were 164.7 and 56.9°C (328.4 and 134.5°F) in dry RH baking that averaged 2.3% relative humidity, whereas in the moist RH baking experiment, these values averaged 140.8 and 89.4°C (285.5 and 192.9°F) and 20.3%, respectively. Opening the oven to remove samples at the halfway point temporarily reduced air temperature and relative humidity, but internal temperature of the bun remained stable.

Changes in *Salmonella* populations

In the dry RH baking experiment, the *Salmonella* counts in inoculated bun dough and dried sesame seeds were on average 7.47 ± 0.68 and 6.13 ± 0.79-log CFU/g, respectively *(Table 4)*. The relatively large standard deviations for the pooled data were the result of a lower inoculum level for Trial 1 vs. Trial 2. *Salmonella* populations (log CFU/g) in dough ranged from 6.78 to 6.97 for T-1 and 8.03 to 8.11 for T-2, whereas for dried sesame seeds ranges were 5.37–5.52 for T-1 and 6.61–6.97 for T-2. Topping the proofed dough buns with 1.5 g inoculated seeds did not result in detectable changes in the *Salmonella* populations in the composite sample. At the halfway baking point (3.5 min, internal temperature 46.5°C, 115.7°F), the *Salmonella* populations in composite seeded buns decreased by an average of 0.9 log units, which might be attributed to the high surface temperature (138.1°C). At the

Table 4. Populations of *Salmonella* (log CFU/g) during baking in oven at dry and moist oven relative-humidity (≤ 3 and average 20% RH, respectively).

Product	Bun baked at ≤ 3% oven RH	Bun baked at avg. 20% RH
Bun Dough	7.5 ± 0.7 [a]	8.5 ± 0.2[a]
Proofed Dough	6.6 ± 1.2 [a]	8.2 ± 0.2[a]
Seeded Dough	6.6 ± 1.2 [a]	8.3 ± 0.1[a]
Half-baked Bun	5.7 ± 0.9 [a]	<1.78[b]
Full-baked Bun	<1.78†[b]	<1.78[b]
Sesame Seed	6.1 ± 0.8[a]	7.5 ± 0.1[a]
Seeds Topped on Half-baked Bun	3.9 ± 0.5[b]	<1[b]
Seeds Topped on Full-baked Bun	2.5 ± 0.4[b] (4/6)^	<1[b]
Half-baked Seeds Alone	Na*	<1[b]
Full-baked Seeds Alone	Na	<1[b]

Data are expressed as mean ± standard deviation of the two replications (n = 6).
Means followed by the lowercase letters in the same column within each relative-humidity treatment are not significantly different ($P \geq 0.05$).
† *Salmonella* colonies not detected by direct plating in lowest dilutions.
^ *Salmonella* colonies recovered from only four out of total six samples.
*Na, Not analyzed.

Table 5. Background microbial populations (log CFU/g) recovered in PCA, APT and PDA plates from products baked at dry and moist oven relative-humidity (≤ 3 and average 20% RH, respectively).

Product	Bun baked at ≤ 3% oven RH			Bun baked at avg. 20% RH		
	PCA	APT	PDA	PCA	APT	PDA
Bun Dough	7.6 ± 0.1	7.8 ± 0.1	7.7 ± 0.2	8.6 ± 0.2	8.7 ± 0.2	8.1 ± 0.1
Proofed Dough	Na*	Na	Na	8.4 ± 0.2	8.4 ± 0.2	7.8 ± 0.2
Seeded Dough	8.1 ± 0.2	8.1 ± 0.7	8.0 ± 0.1	8.5 ± 0.1	8.4 ± 0.1	8.0 ± 0.2
Half-baked Bun	6.8 ± 0.1	6.6 ± 0.1	6.8 ± 0.1	2.2 ± 0.1	<1.78†	<1.78
Full-baked Bun	<1.78	<1.78	<1.78	1.8 ± 0.3	<1.78	<1.78

Data are expressed as mean ± standard deviation of the two replications (n = 6).
*Na, Not analyzed.
† Populations in lowest dilution less than accurate detection limit.

end of baking (7 min; oven temperature setting of 176.7°C; 350°F with RH 2.3 ± 0.9%), the populations in the composite samples were below the detection limit (< 1.78 log CFU/g), representing ≥ 5 log reduction of *Salmonella*. Greater survival of *Salmonella* was detected in sesame seeds isolated from the buns, even though the surface temperature increased to > 73.9°C (165°F) in just 15 sec of baking. An average 3.9 ± 0.5 and 2.5 ± 0.4-log CFU *Salmonella*/g were recovered after 3.5 and 7 min of baking at RH 2.3 ± 0.9%, representing an average 2.2- and 3.6-log reduction at the midpoint and end of baking. The increased thermal resistance of *Salmonella* on the seeds is likely attributed to the low water activity of the seeds (0.269) and the dry heating environment (≤ 3.0% RH) in the oven. In a similar study, Torlak and others *(28)* studied the survival of *Salmonella* in sesame seeds during roasting at 150°C in a forced-air oven. They observed only 2.9-log reduction in 10 min and it required 20 and 30 min to achieve 4.4- and > 5.9-log reductions in *Salmonella* counts, respectively. Lathrop et al., reported a 4.8-log decrease in peanut butter cookies baked at 177°C for 10 minutes *(16)*.

For the moist RH baking experiment, bun dough and dried seed samples were inoculated with 8.5-log CFU (range 8.4–8.7 and 8.3–8.6 for T-1 and T-2, respectively) and 7.5-log CFU/g (range 7.4–7.5 and 7.5–7.6 for T-1 and T-2, respectively). In contrast to the results of the dry RH baking experiment, populations of *Salmonella* in the composite samples, seeds isolated from the bun surface, and seeds alone, decreased to less than detectable levels at the half-bake point (4.5 min) when oven temperature was 204.4°C (400°F), surface temperature ≤ 121°C, an average 20% relative humidity was maintained, and the internal temperature of the bun reached an average of 70.6°C (159°F). Likewise, no *Salmonella* were detected by direct plating at the end of baking. The greater inactivation after wet bulb spike compared to dry conditions has been reported for other foods, such as jerky and beef roast *(7, 25)*.

Baking buns at 204.4°C (400°F) for 9 min with 20.3 ± 8.8% oven humidity, to yield an internal temperature of ≥ 96.7°C (206°F), is sufficient to achieve ≥ 6.0-log reduction of *Salmonella* populations in both the buns and the seed topping at the mid-point of baking even though the bun was underbaked (as determined by the color standard provided by the commercial bakery). This approach demonstrates that the additional times required for baking the product to the desired bun color and product quality provides an additional margin of safety.

While the wet RH baking conditions tested in this study were sufficient to inactivate > 6.0 log *Salmonella* on sesame seeds on the bun at the mid-point, *Salmonella* survivors were detected in four out of six seed samples isolated from the bun surface baked under dry RH conditions, representing approximately a 3.6 log reduction. These findings are significant in the case of consumer home baking, because consumer ovens are not designed to control or achieve a set oven humidity.

Nevertheless, baking the bread dough to a minimum of 89°C will yield product of desired quality, as well as a minimum 5-log reduction in *Salmonella* *(Tables 2–4)*. This study demonstrates that maintaining relative humidity at an average of 20% during baking enhances the lethality on the product surface compared to ≤ 3% RH, and humidity control may therefore be required for products that have toppings, such as seeds, that may be contaminated with *Salmonella*. Regardless of humidity, both methods of baking brought about a ≥ 5 log reduction of *Salmonella* populations, providing adequate validation support for the baking step in a HACCP plan for bread.

Changes in indigenous microflora populations

The reduction in populations of background microflora in samples during baking is presented in *Table 5*. In the dry RH baking method, MRS, PCA, and PDA counts in the buns were reduced by only 0.8 to 1.2-log at half-bake (3.5 min), but populations were below levels detectable by direct plating (< 2-log CFU/g) in samples after full baking (7 min). In the moist baking method, ≥ 5 log reductions in background microbial populations were observed in 4.5 min baking (halfway point). Sporadic colonies recovered from PCA plates from the 9 min baked samples were Gram-positive, rod shaped bacteria (likely *Bacillus* spp., but not further characterized) and not *Salmonella* or yeasts.

When the populations of (added) bread yeasts are compared with those of *Salmonella* during each baking process, the log reduction was similar between the two microbes. The results suggest that the yeasts added to the bread at high populations could serve as an indicator organism to assess the lethality of *Salmonella* in dry and moist baking processes of breads. However, the use of yeasts as a surrogate for organisms in certain toppings, such as sesame seeds, may not be appropriate, because indigenous yeast populations are low and unlikely to be consistent from lot to lot. Furthermore, because the comparison of inactivation rates was limited to only two processes and a single matrix, additional process/matrix combinations should be further investigated to determine if yeast is an appropriate surrogate for *Salmonella* in routine baking validations.

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