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Validation of a nut muffin baking process and thermal resistance characterization of a 7-serovar *Salmonella* inoculum in batter when introduced via flour or walnuts

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ABSTRACT

This study was conducted to validate a commercial nut muffin baking process and to compare the survival of a 7-serovar *Salmonella* cocktail when contaminated via inoculated flour or walnuts. Enriched wheat flour or walnut pieces were mist inoculated with the *Salmonella* cocktail and dried back to the pre-inoculation weight, resulting in a *Salmonella* population level of 6.9 and 8.4 log CFU/g, respectively. Nut muffin batters were prepared separately using inoculated flour or walnuts, followed by baking at 375 °F (190.6 °C) oven temperature for 21 min and post-bake ambient air-cooling (B + C). During baking, > 5-log CFU/g reductions in the *Salmonella* population in nut muffins was achieved in 17 min, and *Salmonella* was not detected by direct plating (< 0.2 log CFU/g detection limit) but was recovered by enrichment at the end of 21 min of baking and B + C. In a separate baking study using an extended baking time (24 min) at 375 °F, *Salmonella* was detected after 24 and 22 min using enrichment plating of nut muffins prepared from inoculated flour and walnuts, respectively. The D-values of the *Salmonella* cocktail in nut muffin batters prepared from inoculated flour were 24.0, 4.0 and 0.6 min at 60, 65 and 70 °C; whereas, corresponding D-values in batters prepared from inoculated walnuts were 22.0, 3.6 and 1.7 min. The *z*-values of the *Salmonella* cocktail in nut muffin batters were 6.1 and 9.0 °C for inoculated flour and walnuts, respectively. This simulated commercial nut muffin baking study utilizing an oven temperature of 190.6 °C for at least 17 min validates that the process will eliminate *Salmonella* populations by ≥5 log CFU/g if pre-baking contamination occurs via flour or walnut ingredients.

1. Introduction

In the United States, *Salmonella* causes one million foodborne illnesses with 19,000 hospitalizations and 380 deaths annually (CDC, 2017). Low water activity (a_w) ingredients, such as flour, nuts, chocolate, and milk and egg powders used in the bakery industry can become contaminated with *Salmonella*. Moreover, *Salmonella* can survive in desiccated conditions for extended periods of time developing greater resistance to heat and various antimicrobial treatments (Beuchat et al., 2013; Finn et al., 2013). In recent years, *Salmonella* has been implicated in several outbreaks and recalls associated with bakery products (Eagle, 2016; Harris, 2016; Marler, 2011). Therefore, it is important to utilize validated baking processes to ensure *Salmonella* free finished food products. Moreover, the U.S. Food and Drug Administration's Food Safety Modernization Act (FSMA) mandates that food processors scientifically validate their processing steps deemed critical in preventing and/or controlling food safety hazards (FDA, 2017).

In a previous commercial baking simulation study for the manufacture of plain muffins (Channaiah et al., 2017), we demonstrated a ≥ 5-log cycle *Salmonella* population reduction within 17 min using a 190.6 °C oven temperature. In this previous study, a 3-serovar *Salmonella* cocktail contamination was introduced via flour to prepare plain muffins; whereas, in the current research, we studied the survival of a 7-serovar *Salmonella* cocktail during muffin baking when an additional low a_w and higher fat ingredient, walnut pieces, was included in the recipe. The *Salmonella* contamination was introduced via inoculated

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flour or walnuts. Additionally, the thermal resistance parameters (D- and z-values) of the 7-serovar *Salmonella* cocktail during heating of the nut muffin batter were determined.

2. Materials and methods

2.1. Salmonella culture propagation and inoculum preparation

Three *Salmonella enterica* serovars [Senftenberg 775 W (ATCC 43845), Newport (ATCC 6962) and Typhimurium (ATCC 14028)] were obtained from the American Type Culture Collection (ATCC®, Manassas, VA), whereas *S*. Tennessee and three non-typed *Salmonella* isolates from dry pet food manufacturing environments were obtained from Richter International, Inc. (Columbus, OH). All cultures were individually stored at −80 °C on protective beads in glycerol (Microbank™ Bacterial and Fungal Preservation System, Pro-Lab Diagnostics, Round Rock, TX). The cultures were individually activated by transferring one bead of a culture into 10 mL brain heart fusion (BHI; Becton, Dickinson and Company, Sparks, MD) broth and incubating at 37 °C for 24 h. These activated cultures were confirmed using API® 20E (bioMérieux, Inc., Durham, NC) and stored at 4 °C for use during experiments. The 7-serovar *Salmonella* cocktail was prepared as described by Channaiah et al. (2017) by harvesting 24-h *Salmonella* serovar lawns grown on Brain Heart Infusion (BHI) agar using 0.1% peptone (Becton, Dickinson and Company, Sparks, MD) solution, followed by combining the solutions to generate a cocktail master inoculum comprised of approximately equal ratios of each serovars.

2.2. Inoculation of flour and walnut pieces

The enriched wheat flour (King Midas Special, ConAgra Mills, Omaha, NE) and walnut pieces (Debittered Walnuts, American Almond Products Co., Brooklyn, NY) were inoculated with the 7-serovar *Salmonella* cocktail using the method described by Channaiah et al. (2017). The flour (375 g) or walnut pieces (200 g) were weighed into a sanitized sealable plastic tub (9.4 L, Rubbermaid, Atlanta, GA) and spread uniformly into a thin layer. With the tub placed into a large biohazard bag inside a large biosafety cabinet, the inoculum was evenly misted onto the ingredient surfaces (~3 mL onto flour and ~2 mL onto walnut pieces), the tub lid sealed, and the contents mixed well by manually shaking the sealed tub for ~30 s. The external surfaces of the tub was disinfected and removed from the biosafety cabinet, and the lid was removed and tub was placed into a 37 °C incubator (Lab-Line®, Imperial III Incubator, Melrose Park, IL) for ~5 h to dry back to the original pre-inoculation weight. This was done by verifying that the weight of inoculated, dried flour or walnuts was similar to the pre-inoculation weight. Tub was resealed with lid and the inoculated, dried flour or walnut pieces were mixed again, and stored at ambient temperature (~25 °C) for use to make nut muffin batters within 7 days.

2.3. Baking optimization to mimic an industry standard process

To optimize the laboratory nut muffin baking process to approximate a standard industry process, a series of preliminary trials were conducted to determine the optimum baking temperature and time. The nut muffins baked at 375 °F (190.6 °C) oven temperature for 21 min were found to match the commercial baking industry's standard nut muffin end-use quality parameters viz., crust color, appearance, size, moistness and texture.

2.4. Nut muffin batter preparation and baking

The ingredients and recipe (ingredients table provided as supplementary material) used in this study were provided by AIB International, Inc. (Manhattan, KS) and represented a standard commercial nut muffin product with 75 g of walnuts used per batch (Channaiah et al., 2017). Batters were prepared using inoculated flour or walnut pieces according to Channaiah et al. (2017). Briefly, 70 ± 0.5 g of inoculated batter was placed into each chamber of a 12-chamber muffin pan (3-cm height, and 5-cm bottom and 8-cm top diameter of each chamber) lined with parchment muffin cups. The pan was transferred to a pre-heated kitchen oven (Whirlpool® FlexHeat™ Dual Radiant Element; Benton Harbor, MI) and baked at 190.6 °C oven temperature for 21 min. The temperatures of oven and muffins were continuously monitored using an eight-channel data logging system (USB-TC with MCC DAQ software, Measurement Computing, Norton, MA) and fine-gauge type T thermocouples (Omega Engineering Inc., Stamford, CT). During the baking process, muffins were sampled in duplicates by randomly removing two muffins (~60 g each) from the pan [at 13, 15, 17, 19 and 21 min, and after 30 min post-baking ambient cooling (B + C)], and then individually mixed into 150 mL chilled 0.1% peptone buffer, which resulted in the initial dilution factor of 3.5 in each stomacher bag. The samples were stomached for 1 min, serially diluted using 0.1% peptone buffer, and *Salmonella* populations were enumerated by spread plating 0.1 mL dilutions in duplicates. However, the initial/stomacher bag dilutions were plated just using 1 mL distributed on three plates. The final *Salmonella* populations were calculated by averaging the *Salmonella* counts of duplicate muffins sampled at each sampling point. Therefore, plating the lowest dilution (stomacher bags) according to the used plating scheme resulted in the detection limit of 0.2 Log CFU/g. The aforementioned sampling points were selected based on the preliminary work (data not presented) that demonstrated significant increase in muffin temperatures (> 80 °C) and decrease in *Salmonella* populations. The surviving *Salmonella* population at each time interval was determined by spread plating using injury-recovery [BHI agar plates overlaid with xylose lysine deoxycholate (XLD) after 5 h of incubation (XLD; Becton, Dickinson and Company)] and selective XLD agar plates (Channaiah et al., 2017).

To determine the baking time (breakpoint) at which no viable *Salmonella* was detected in the nut muffins, an additional inoculated study was conducted using an extended baking time (up to 24 min). Nut muffins were sampled and plated at 19, 20, 21, 22, 23 and 24 min. For the sampling points at which no visible *Salmonella* colonies were detected on agar plates, 25 g of the original homogenized samples (retained in refrigerated storage) were mixed with 225 mL of BHI broth, incubated at 37 °C for 24 h, and streak plated on XLD agar followed by incubation at 37 °C for 24 h to qualitatively detect viable *Salmonella* levels below the detection limit (0.2 log CFU/g) of the direct plating protocol.

To profile the a_w and pH of the nut muffins during the baking process, batters were prepared using non-inoculated ingredients, and sampled at 3, 6, 9, 12, 15, 18 and 21 min of baking, and B + C (Channaiah et al., 2017). Muffin pH was measured at 25 °C by mixing 10 g of muffin (crumb + crust) in 90 mL deionized water, and using calibrated pH meter (Corning Pinnacle, 530 pH meter, Corning Inc., Corning, NY). For a_w determination, muffin crumb and crust were separated, transferred to sealed a_w cups (Decagon Devices, Inc., Pullman, WA), cooled to ~25 °C, and a_w measured using AquaLab Dewpoint 4TE a_w meter (Decagon Devices, Inc.). Additionally, non-inoculated nut muffins were obtained after 15, 18 and 21 min of baking, and B + C to analyze for proximate composition. Samples for proximate analyses were stored in airtight plastic bags at ~4 °C and were sent to the Animal Sciences and Industry department's analytical laboratory (Weber Hall, Kansas State University, Manhattan, KS).

2.5. Thermal inactivation parameters

D-values of the 7-serovar *Salmonella* cocktail in nut muffin batters prepared from inoculated flour or walnut pieces were determined using thermal-death-time disks in heated circulating water baths at 60, 65 and 70 °C using sampling times of 20, 5 and 0.5 min, respectively, according to the method described by Michael et al. (2014) and

Channaiah et al. (2017). At each time-temperature point, *Salmonella* population survival was enumerated by spread plating on injury-recovery media. The D- and *z*-values were calculated for individual replications using the linear regression graphs plotted in Microsoft Excel 2011 (Microsoft Corp. Redmond, WA), and the final D- and *z*-values were calculated by averaging the three replication values.

2.6. Statistical analyses

The baking and thermal inactivation parameters were independent studies, and utilized randomized complete block designs with three replications as blocks. The *Salmonella* populations, pH and a_w from the baking study, and D- and *z*-values were analyzed by one-way analysis of variance (ANOVA) using SAS version 9.3 (SAS Institute, Cary, NC), and $P \leq 0.05$ was considered significant.

3. Results and discussion

The mean internal nut muffin temperature at the start of baking was ~22 °C, which increased to ~70 °C at 12 min and 100 °C at 19.8 min during oven baking (temperature profile figure provided as supplementary material). The internal temperature of nut muffins was ~101 °C at the end of 21 min of baking, and remained > 70 °C for the first 8.5 min of ambient air cooling. Ultimately, the internal muffin temperature decreased to ~39 °C at B + C. Compared to the temperature profile of plain muffins previously reported by Channaiah et al. (2017) using the same baking process parameters, the rate of increase in temperature during baking and rate of decrease in temperature during ambient cooling of nut muffins was slightly slower; however, both plain and nut muffins demonstrated similar start, maximum and B + C temperatures.

The initial pH of nut muffin batter was 6.50 ± 0.21 and increased to 7.30 ± 0.02 in the fully baked and cooled nut muffins (pH graph provided as supplementary materials). The a_w of nut muffin batter (0.925 ± 0.001) increased to 0.937 ± 0.004 in nut muffin crumb at the end of 21 min of baking but decreased back to 0.925 ± 0.001 at B + C (a_w graph provided as supplementary materials). The crust of nut muffins started to notably dry compared to the crumb by 12 min of baking, and the a_w of the crust was significantly lower than that of crumb for the remainder of the baking and cooling period. The final a_w of nut muffin crust was 0.701 ± 0.034. Channaiah et al. (2017) reported a pH of 7.52, and crumb and crust a_w readings of 0.928 and 0.700, respectively, for plain muffins at B + C. These values were very similar to readings obtained for nut muffins in the current study. The proximate analyses of nut muffin batter and nut muffins during baking and at B + C are provided as supplementary materials. As expected, the fat, protein and starch contents of baked, cooled nut muffins were greater than plain muffins reported by Channaiah et al., 2017.

The 7-serovar *Salmonella* inoculum prepared from agar plate lawns contained ~11 log CFU/mL of *Salmonella*. The initial *Salmonella* populations (as enumerated using injury-recovery plating) in the inoculated, dried flour and walnut pieces were 6.9 ± 0.27 and 8.4 ± 0.05 log CFU/g, respectively (Fig. 1A and B). Although approximately equal proportions of *Salmonella* cocktail were used to inoculate flour and walnuts, the greater *Salmonella* population in walnut pieces compared to flour could be due to better adsorption of inoculum onto the walnut surfaces and/or due to a protective effect of the higher lipid nuts during re-drying (Nascimento et al., 2018). *Salmonella* populations in batter prepared from inoculated flour and nuts were 7.0 ± 0.27 and 7.4 ± 0.06 log CFU/g, respectively. For nut muffins prepared from inoculated flour, *Salmonella* population decreased to 0.3 ± 0.10 log CFU/g at 19 min of baking (> 6 log cycle reduction from original population in pre-baked batter), and were < 0.2 log CFU/g by the end of 21 min of baking and B + C (Fig. 1A). For the nut muffins prepared from inoculated walnut pieces, *Salmonella* population decreased to 0.4 ± 0.10 log CFU/g at 17 min (> 7 log cycle reduction from original population in pre-baked batter), and were < 0.2 log CFU/g at 21 min and B + C (Fig. 1B). For the breakpoint determination or the time at which no viable *Salmonella* was recovered by enrichment plating, *Salmonella* was detected in the nut muffins prepared from inoculated flour for up to 24 min of baking compared to 22 min in the nut muffins prepared from inoculated walnuts. Similar to the plain muffins (Channaiah et al., 2017), > 5-log *Salmonella* reductions were attained in the nut muffins at 17 min of baking. However, *Salmonella* could be detected in plain muffins at a level of 0.8 log CFU/g (Channaiah et al., 2017) compared to < 0.2 log CFU/g in the current study. This difference could possibly be attributed to the make-up of the *Salmonella* cocktails (3-serovars versus 7-serovars) used in the two studies, and differences in processing parameters (heating and cooling rates) and proximate composition attributed to the added nuts into the muffin formulation.

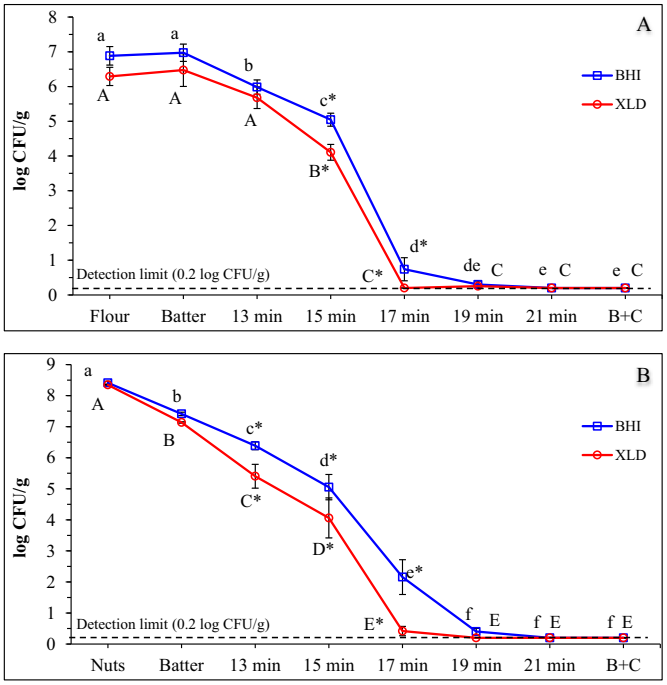

Fig. 1. Mean (± SE) survival of a 7-serovar *Salmonella* cocktail in nut muffins prepared from inoculated flour (A) or walnut pieces (B) during 21 min of baking at 190.6C oven temperature followed by 30 min of ambient air cooling (B + C). a–f and A–F: *Salmonella* recovery values within a defined culture medium [injury recovery (BHI) or selective (XLD)] with different letters are significantly different across baking times ($P \leq 0.05$). *: *Salmonella* recovery values at a given sampling time are significantly different ($P \leq 0.05$) between culture media types.

The calculated mean D- and *z*-values along with R^2 values are presented in Table 1. The *z*-values and D-values at 60 and 65 °C of the *Salmonella* cocktail in nut muffin batters prepared from inoculated flour

Table 1
Mean (± SE) D-values (min) and *z*-values (°C) of a 7-serovar *Salmonella* cocktail in nut muffin batter prepared from inoculated flour or walnut pieces.

	Inc. flour		Inc. walnuts	
	Values	R^2	Values	R^2
D_{60}	24.0 ± 3.18[a]	0.9402	22.0 ± 1.89[a]	0.9863
D_{65}	4.0 ± 0.87[a]	0.9946	3.6 ± 0.54[a]	0.9874
D_{70}	0.6 ± 0.13[a]	0.9853	1.7 ± 0.29[b]	0.999
z	6.1 ± 0.53[a]	0.9993	9.0 ± 0.94 [a]	0.9502

[a–b]Values within a row with different superscripts are significantly different ($P \leq 0.05$).

or walnuts were similar ($P > 0.05$); however, the *Salmonella* D-value at 70 °C in nut muffin batter prepared from inoculated walnuts was greater than the D-value in batter prepared from inoculated flour (Table 1). This greater *Salmonella* D-value at 70 °C in muffin batter prepared with inoculated walnuts compared to muffin batter prepared from inoculated flour could be due the better protective effect of fat in nuts on *Salmonella* cells at higher temperatures. Channaiah et al. (2017) reported that D_{55}, D_{58} and D_{61} values in plain muffin batter were 62.2, 40.1 and 16.5 min, respectively, and the *z*-values was 10.4 °C. This slight difference in *z*-values of *Salmonella* observed in nut muffin batters compared to plain muffin batter could be attributed to the greater fat and protein contents in the nut muffin batters (due to the walnuts as an additional ingredient) compared to the plain muffin batter. Moreover, the differences in the *Salmonella* cocktails used in the current study and by Channaiah et al. (2017) could result in the slightly different D- and *z*-values. During D-values studies at 70 °C treatment, *Salmonella* populations in nut muffin batter decreased < 4 log CFU/g by the time target temperature of 70 °C was achieved inside the TDT disks. This lower *Salmonella* population at the start of thermal treatment at 70 °C resulted in a quick decline in *Salmonella* population and very short sampling time. These factors could increase the experimental error and should be avoided in future experiments by using lower temperatures and longer sampling times to calculate more accurate D-values.

4. Conclusions

This study validated that a baking process simulating commercial manufacturing of nut muffins can be expected to achieve ≥5-log CFU/g reductions in *Salmonella* populations by ≥17 min using a ≥ 190.6 °C oven temperature, irrespective of whether the *Salmonella* contamination originates in the raw flour or walnut pieces used as batter ingredients. Channaiah et al. (2017) reported similar observations for plain muffins prepared using the same baking process. However, in D-value studies using temperatures lower than oven baking temperatures, *Salmonella* exhibited slightly greater heat resistance in the nut muffin batters. This data can be used by nut muffin manufacturers to validate their baking processes; however, consideration should be given to the impact of different commercial oven performance characteristics, alternative time-temperature baking schedules, and to any muffin formulation differences than those used in the current study.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2019.01.013.

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