International Journal of Food Microbiology 250 (2017) 1–6

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

Validation of the baking process as a kill-step for controlling *Salmonella* in muffins

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ARTICLE INFO

Article history:
Received 14 July 2016
Received in revised form 23 January 2017
Accepted 11 March 2017
Available online 12 March 2017

Keywords:
Muffin
Salmonella
Food safety
Quick bread

ABSTRACT

This research investigates the potential risk of *Salmonella* in muffins when contamination is introduced via flour, the main ingredient. Flour was inoculated with a 3-strain cocktail of *Salmonella* serovars (Newport, Typhimurium, and Senftenberg) and re-dried to achieve a target concentration of ~8 log CFU/g. The inoculated flour was then used to prepare muffin batter following a standard commercial recipe. The survival of *Salmonella* during and after baking at 190.6 °C for 21 min was analyzed by plating samples on selective and injury-recovery media at regular intervals. The thermal inactivation parameters (D and *z* values) of the 3-strain *Salmonella* cocktail were determined. A ≥5 log CFU/g reduction in *Salmonella* population was demonstrated by 17 min of baking, and a 6.1 log CFU/g reduction in *Salmonella* population by 21 min of baking. The D-values of *Salmonella* serovar cocktail in muffin batter were 62.2 ± 3.0, 40.1 ± 0.9 and 16.5 ± 1.7 min at 55, 58 and 61 °C, respectively; and the z-value was 10.4 ± 0.6 °C. The water activity (a_w) of the muffin crumb (0.928) after baking and 30 min of cooling was similar to that of pre-baked muffin batter, whereas the a_w of the muffin crust decreased to (0.700). This study validates a typical commercial muffin baking process utilizing an oven temperature of 190.6 °C for at least 17 min as an effective kill-step in reducing a *Salmonella* serovar population by ≥5 log CFU/g.

1. Introduction

The presence of *Salmonella* in bakery products has been a concern for many years (Lathrop et al., 2014). Studies have indicated that microorganisms, including *Salmonella* can survive in a latent state for extended periods in wheat flour, and can emerge from dormancy when favorable conditions prevail, such as in batter or mixes (Eglezos, 2010). Low levels of *Salmonella* contamination of flour and flour-based mixes have been the cause of numerous foodborne *Salmonella* outbreaks (Berghofer et al., 2003; FDA, 2005, 2015b; New Zealand Food and Safety Authority, 2008; Richter et al., 1993; Sperber, 2007; Zhang et al., 2007). Additionally, raw ingredients such as flour, sugar, milk powders, yeast, chocolate, cocoa powder, nuts and peanut butter used in the manufacturing of bakery products can carry pathogens and result in pre-baking microbial contamination (Akins, 2014; GMA, 2009a, 2009b; Saranraj and Geetha, 2012). In the U.S., bakery products have been associated with 142 foodborne disease outbreaks and 2822 illnesses from 2004 to 2013 (CSPI, 2015). Among *Salmonella* spp., serotypes Enteritidis, Typhimurium, Heidelberg, Newport and Javiana were most often implicated in foodborne disease outbreaks (CDC, 2015). Depending upon the age and health condition of an individual, the infectious dose for the *Salmonella* infection can be as low as one cell (FDA, 2012).

The Food Safety Modernization Act (FSMA), which was enacted in 2011, requires that food manufacturers should establish and implement a food safety system using hazard analysis and risk-based preventive controls (HARPC) (FDA, 2015a, 2016). HARPC requires validation and verification of all preventive controls that are critical processing steps for food safety by obtaining and evaluating scientific and technical evidence to determine whether the preventive controls, when properly implemented, will effectively control the hazards that are reasonably likely to occur (FDA, 2016).

Although ingredients such as flour, chocolate, peanut butter, dairy products, spices, etc. can be potential sources of *Salmonella*, the majority of outbreaks involving bakery goods have been associated with eggs, and in most cases, the incidents were due to under-baking (Lathrop et

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http://dx.doi.org/10.1016/j.ijfoodmicro.2017.03.007

al., 2014). While baking is considered to be an effective kill-step in controlling foodborne pathogens in bakery products, formal scientific validation of the diversity of commercial baking processes for the inactivation of pathogens has not been thoroughly studied (Lathrop et al., 2014; Lopez, 2014). This scientific documentation is necessary to meet validation requirements of HARPC under the FSMA regulations.

In a previous study, we demonstrated that a typical hamburger bun baking process utilizing oven temperatures ≥218.3 °C (425 °F) was efficient in reducing high levels (>6 log CFU/g) of *Salmonella* Typhimurium, Newport and Senftenberg 775W (Channaiah et al., 2016). It is important to understand how *Salmonella* serovars behave in a multitude of baked products, as these are characterized by highly variable intrinsic properties and manufacturing protocols. Therefore, in this study, we chose to validate the baking process of a basic plain muffin due to its distinct characteristics: chemically leavened with high moisture, fat and sugar contents and relatively low baking temperature. The objectives of this study were: (1) validate a simulated commercial muffin manufacturing process that features a 21 minute bake time at 190.6 °C (375 °F) oven temperature to control a 3-serotype cocktail of *Salmonella*, and (2) determine thermal inactivation parameters (D- and *z*-values) for the 3-serovar *Salmonella* cocktail in muffin batter. Additionally, pH and water activity (a_w) values in muffins were determined at regular time intervals during baking.

2. Materials and methods

2.1. Design of studies and statistical analysis

This study was designed as a randomized complete block (three replications as blocks) with seven treatments (inoculated flour and batter; 13, 15, 19 and 21 min of baking; and baking for 21 min followed by 30 min of ambient cooling (B + C). All analyses were done in duplicate. All activities involving inoculated flour/batter preparation, baking and *Salmonella* enumeration were conducted in a Biosafety Level-2 pilot food processing laboratory by following appropriate approved biosafety procedures. *Salmonella* population, pH and a_w and all other experimental data were analyzed using one-way analysis of variance (ANOVA) using SAS version 9.3 (SAS Institute, Cary, NC), and $P \leq 0.05$ was considered significant.

2.2. Salmonella cultures and inoculum preparation

Salmonella enterica serovars Typhimurium (ATCC 14028), Newport (ATCC 6962) and Senftenberg 775W (ATCC 43845) were obtained from the American Type Culture Collection (ATCC; Manassas, VA), propagated according to the manufacturer's instructions, and then stored individually at −80 °C on protectant beads in glycerol (Microbank™ Bacterial and Fungal Preservation System, Pro-Lab Diagnostics, Round Rock, TX). All cultures were re-activated individually by transferring one bead into 10 mL brain heart fusion (BHI; Becton, Dickinson and Company, Sparks, MD) broth, incubated at 37 °C for 24 h, and stored at 4 °C. Individual cultures from BHI broth were streaked onto BHI agar in order to form lawns (eight plates for each *Salmonella* serovar) and incubated at 37 °C for 24 h. Lawns from each plate were harvested by dispensing 1 mL of 0.1% peptone (Becton, Dickinson and Company) solution on agar surfaces twice, dislodging culture growth using disposable L-shaped cell spreaders (Fisherbrand®, Fisher Scientific™, Pittsburg, PA), and pipetting culture solutions of individual serovars into a 50-mL conical tube to provide ~16 mL. The three culture solutions were then mixed in equal proportions in a conical tube to serve as the master inoculum for inoculating flour.

2.3. Flour inoculation

Enriched (niacin, reduced iron, thiamine mononitrate, riboflavin and folic acid) wheat flour (King Midas Special, ConAgra Mills, Omaha, NE; 375 g) was weighed into a sanitized sealable plastic tub (9.4 L, Rubbermaid, Atlanta, GA), spread into an even layer, and the tub placed inside of a biological safety cabinet. The flour was then inoculated with master inoculum by spray misting ~3 mL of the mixed inoculum solution uniformly across the flour layer. The lid was placed onto the tub and the flour was mixed well by shaking inside the biosafety cabinet. Inoculated flour in the tub was then dried to the original pre-inoculation weight inside of an incubator (Lab-Line®, Imperial III Incubator, Melrose Park, IL) at 37 °C for ~5 h with open lid. The dried inoculated flour was stored at ambient temperature (~25 °C) inside of the closed tub until used (within 7 days).

2.4. Muffin batter preparation

The recipe and all ingredients for muffin preparation were provided by AIB International (Manhattan, KS; Table 1). Inoculated (for microbial studies) or non-inoculated (for pH and a_w study) flour and other dry ingredients (granulated sugar, baking powder, salt, nonfat dry milk, whole egg solids and all-purpose shortening) were weighed into a sanitized mixing bowl (Artisan®, KitchenAid®, St. Joseph, MI) and mixed with a spatula. Water was added to the dry ingredients according to the recipe, the mixing bowl and paddle were attached to the mixer, and the batter was mixed for 2 min at low speed. Muffin batter (70 ± 0.5 g) was placed into twelve compartments of a sanitized aluminum muffin pan lined with parchment muffin cups (2 in. × 1¼ in. wax baking cups; Paterson Pacific Parchment Co., Sparks, NV). The pans used had a 3-cm height, and 5-cm bottom and 8-cm top diameters of each muffin chamber.

2.5. Muffin baking and temperature measurement

The electric kitchen oven (Whirlpool®, 4.8 cubic feet, FlexHeat™, Dual Radiant Element, Benton Harbor, MI) was preheated to 190.6C for 30 min prior to use to ensure equilibration, which was confirmed 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). A pan with twelve cups containing raw muffin batter was placed into the oven and two pre-determined random muffins were used to monitor the temperature during 21 min of baking and 30 min of ambient cooling. The oven and internal muffin temperatures were continuously monitored and recorded every second by inserting thermocouples from the top surface into the geometric centers of the two muffins.

2.6. pH, water activity and proximate analyses

For the pH and a_w study, one random muffin was sampled at 3, 6, 9, 12, 15, 18 and 21 min of baking followed by one sample at the end of 30 min of ambient cooling. At each sampling point, a selected muffin was divided longitudinally into two halves: one for the pH and other for the a_w determination. The pH meter (Corning Pinnacle, 530 pH meter, Corning Inc., Corning, NY) was calibrated using 4.0 and 7.0 standard buffer solutions (Ricca Chemical Company®, Arlington, TX), and the total muffin pH was measured at 25 °C by mixing 10 g of

Table 1
Ingredients and recipe used to prepare muffin batter.

Ingredient	Weight (g)
Flour	300
Granulated sugar	180
Baking powder	15
Salt	3.8
Nonfat dry milk	22.5
Whole egg solids	22.5
All-purpose shortening	120
Water	247.5