

Kill Step Validation Model for Bakery Applications

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AIB International



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Outline:

- ❑ **Introduction**
- ❑ **Kill-Step Validation Gen-1:**
 - Foundational research project: Hamburger bun kill-step validation research
 - Baking Process Kill-Step Calculator
- ❑ **Kill-Step Validation Gen-2:**
- ❑ **Expected outcomes**



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Introduction

- ❑ Approximately 2,800 commercial bakeries and 6,000 retail bakeries operate in the U.S., with a market value of nearly \$30 billion/ year (Hussain 2014).
- ❑ The U.S. baking industry comparatively has a very safe record for the production of shelf stable processed foods
- ❑ Microbiological spoilage is often the major factor limiting the shelf life of bakery products leading to huge economic losses (Smith et al. 2010)
- ❑ Although not directly linked to production practices, there were 4,200 illnesses associated with bakery products reported in the U.S. between 1998 and 2011 (CSPI 2009)



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Bakery Products: Food Safety Challenges

- ❑ The presence of *Salmonella* spp. in bakery ingredients and products could create a public health risk if the product is improperly baked
- ❑ Research findings revealed that *Salmonella* can survive in adverse conditions (e.g., low moisture foods) for a long time (Gruzdev et al. 2011 and Eglezos 2010)
- ❑ Pathogens such as *Salmonella* spp. can be introduced into bakery products through a wide range of ingredients such as egg (Board 1969; FSIS 1998), milk products (El-Gazzar et al. 1992; Ahmad et al. 2000), flour (Richter et al. 1993; Dack 1961), milk chocolate (D'Aoust 1977), coconut (Goepfert 1980), peanut butter (Scheil et al. 1998), fruit (Golden et al. 1993), spices (Hara-Kudo et al. 2000) and yeast flavorings (Joseph et al. 1991)



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The Need....

- ❑ Although most bakery products undergo a **putative kill step** at the point of production, such as baking or cooking, there lacks published **scientific proof or validation research**
- ❑ **FDA-FSMA** regulatory requirement requires validation and verification of kill-step

Validation

- ❑ A preemptive scientific evaluation providing documentary evidence that a particular process is capable of consistently delivering a product, meeting its pre-determined specifications
- ❑ A collection of scientific evidence



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Key FSMA Validation Requirement



What would constitute validation?

(§117.160(b)(2)): The proposed rule would require that the validation of preventive controls include collecting and evaluating scientific and technical information or, when such information is not available or is insufficient, conducting studies to determine whether the preventive controls, when properly implemented, will effectively control the hazards that are reasonably likely to occur

- Must be performed (or overseen) by a preventive controls qualified individual; or
- Within 90 calendar days after production of the applicable food first begins; or
- Whenever a change to a control measure occurs; or
- Whenever a reanalysis of the food safety plan reveals the need to do so;



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Hamburger Bun Validation Research

- ❑ We used hamburger bun manufacturing as the model to develop a scientific validation protocol, as it is one of the most popular bakery products consumed in the U.S. and in Canada.
- ❑ This is the first study of its kind involving validating a bakery product with baking as a kill step for *Salmonella* spp., *E. faecium* and *Saccharomyces cerevisiae*



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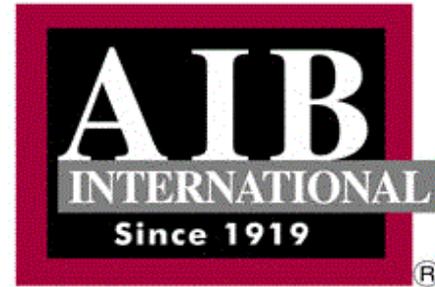
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Research Collaborators



American Bakers Association



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Goal

To validate a simulated commercial baking process for hamburger buns to destroy *Salmonella* spp., and to determine the appropriateness of using non-pathogenic surrogates (*Enterococcus faecium* ATCC 8459 or *Saccharomyces cerevisiae*) for in-plant process validation studies



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Research Objectives

1. Validate the baking process as a kill step in reducing *Salmonella* spp. during the manufacture of hamburger buns
2. Validate the baking step's effectiveness in reducing *S. cerevisiae* and *E. faecium* to determine appropriateness of utilizing non-pathogenic surrogate for industry validation and process verification purposes, and
3. Determine the heat resistance viz, D-value, and z-value of *Salmonella* spp., *S. cerevisiae* and *E. faecium* in hamburger bun dough



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Microorganisms Used

❑ *Salmonella* serovars:

- *Salmonella* enterica serovar Typhimurium (ATCC 14028)
- *Salmonella* enterica serovar Newport (ATCC 6962)
- *Salmonella* enterica serovar Senftenberg (ATCC 43845)

❑ *Enterococcus*:

- *Enterococcus faecium* (ATCC 8459)

❑ *Yeast*:

- *Saccharomyces cerevisiae* (Compressed Yeast)



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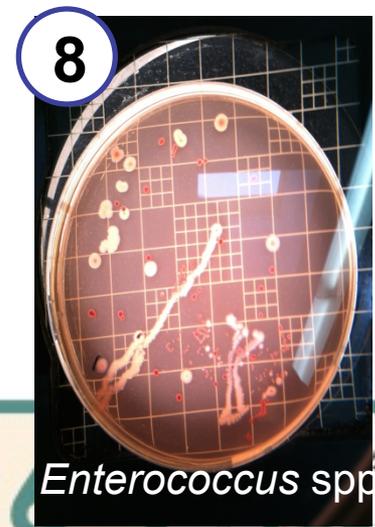
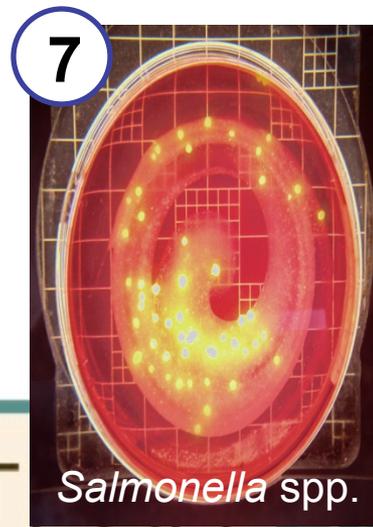


Flour Inoculation

- ❑ Two trays (35.6 cm x 21.6 cm x 14 cm) of flour containing 400 g each were mist inoculated with 4 mL of the target culture to achieve 6-7 log₁₀ CFU/g
- ❑ This inoculation procedure was conducted within a Class II Type A2 biosafety cabinet
- ❑ After 5 min, trays of inoculated flour were placed into a 37°C incubator until they returned to pre-inoculation dry weight
- ❑ Inoculated flour was stored at ambient temperature for 36-48 h prior to use
- ❑ Final target bacterial concentrations of the flour were determined immediately prior to initiation of mixing the dough

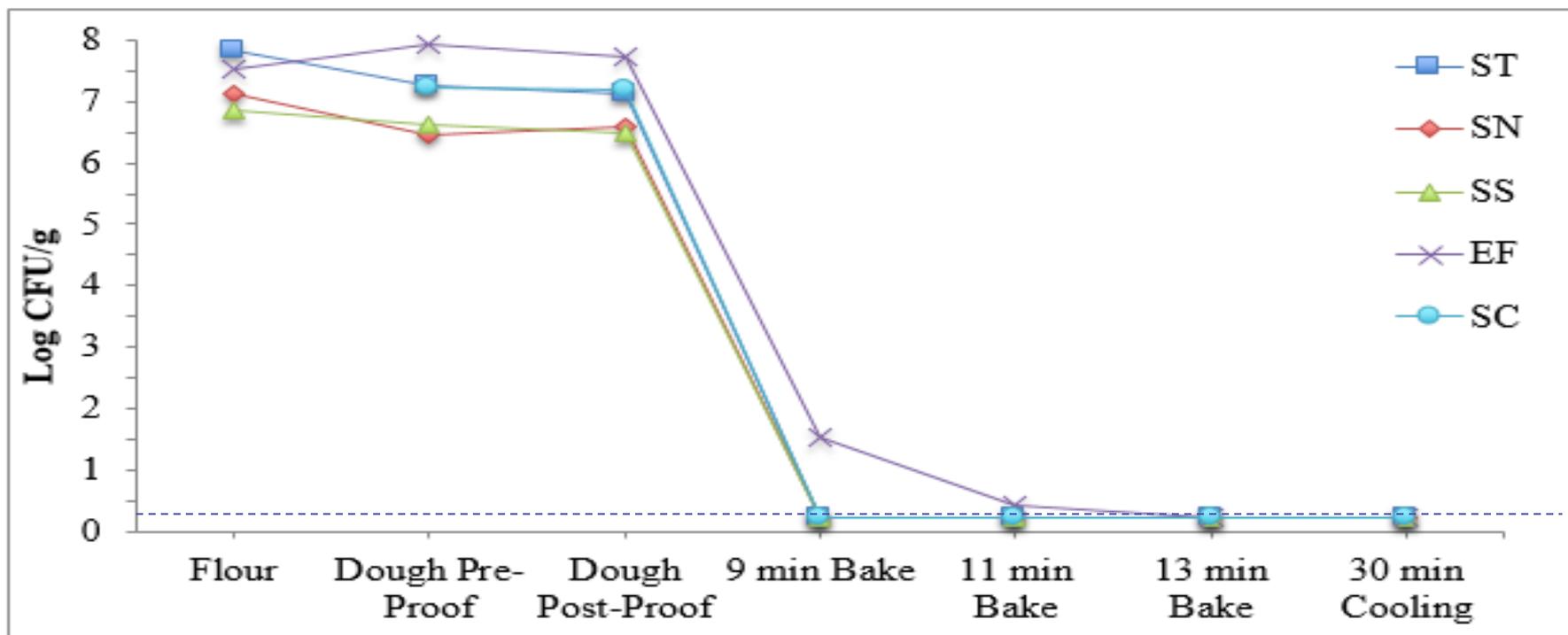


Overview

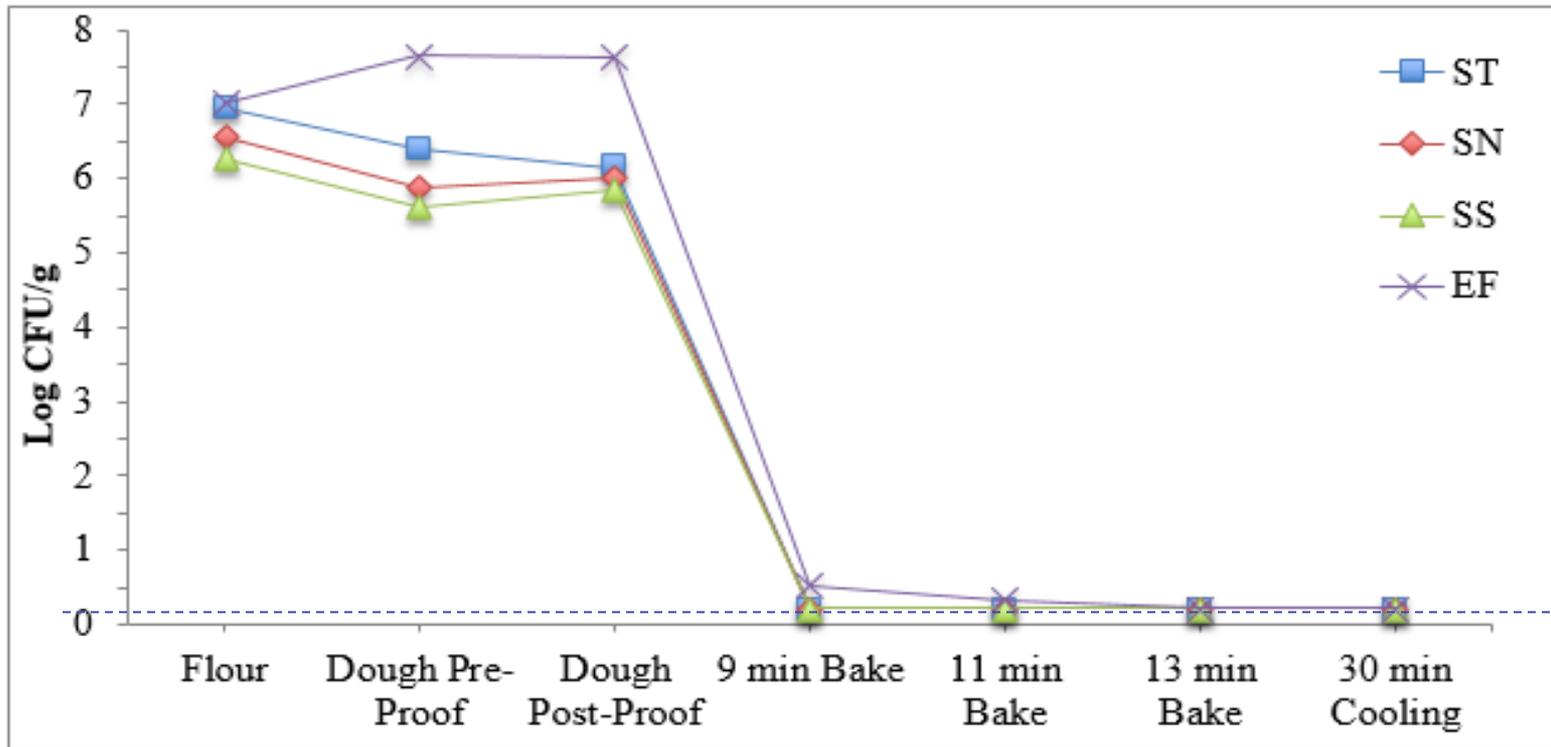


Results

Figure 1. Survival population of *Salmonella* serovars, *Enterococcus faecium* and *Saccharomyces cerevisiae*, in hamburger buns during baking at 218°C (using selective media)



Survival population of *Salmonella* serovars, *Enterococcus faecium* and *Saccharomyces cerevisiae*, in hamburger buns during baking at 218°C



DL



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Microbial Kinetics Study

Salmonella spp.

E. faecium

	BHI/XLD ^a	XLD ^b	BHI/mEA ^c	mEA ^d
Temperature (°C)				
55	28.64 ± 5.19	21.30 ± 2.61	133.33 ± 0	87.21 ± 4.74
58	7.61 ± 0.61	7.53 ± 0.61	55.67 ± 9.0	45.33 ± 6.79
61	3.14 ± 0.32	2.29 ± 0.21	14.72 ± 4.11	6.14 ± 0.47
z-value	6.68 ± 0.94	6.22 ± 0.32	6.25 ± 0.80	5.20 ± 0.05

^a Injury-recovery media, Brain Heart Infusion (BHI) agar with Xylose Lysine Desoxycholate (XLD) agar overlay; ^b Selective medium, XLD agar; ^c Injury-recovery media, BHI agar with m-Enterococcus (mE) agar overlay; ^d Selective medium, mE agar.

Salmonella cocktail: *S. enterica* serovar Typhimurium (ATCC 14028); *S. enterica* serovar Newport (ATCC 6962); *S. enterica* serovar Senftenberg (ATCC 43845)



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D-values

Temperature	Bun dough
52	18.73 ± 0.72
55	5.67 ± 1.51
58	1.03 ± 0.21
z-value	4.74 ± 0.34

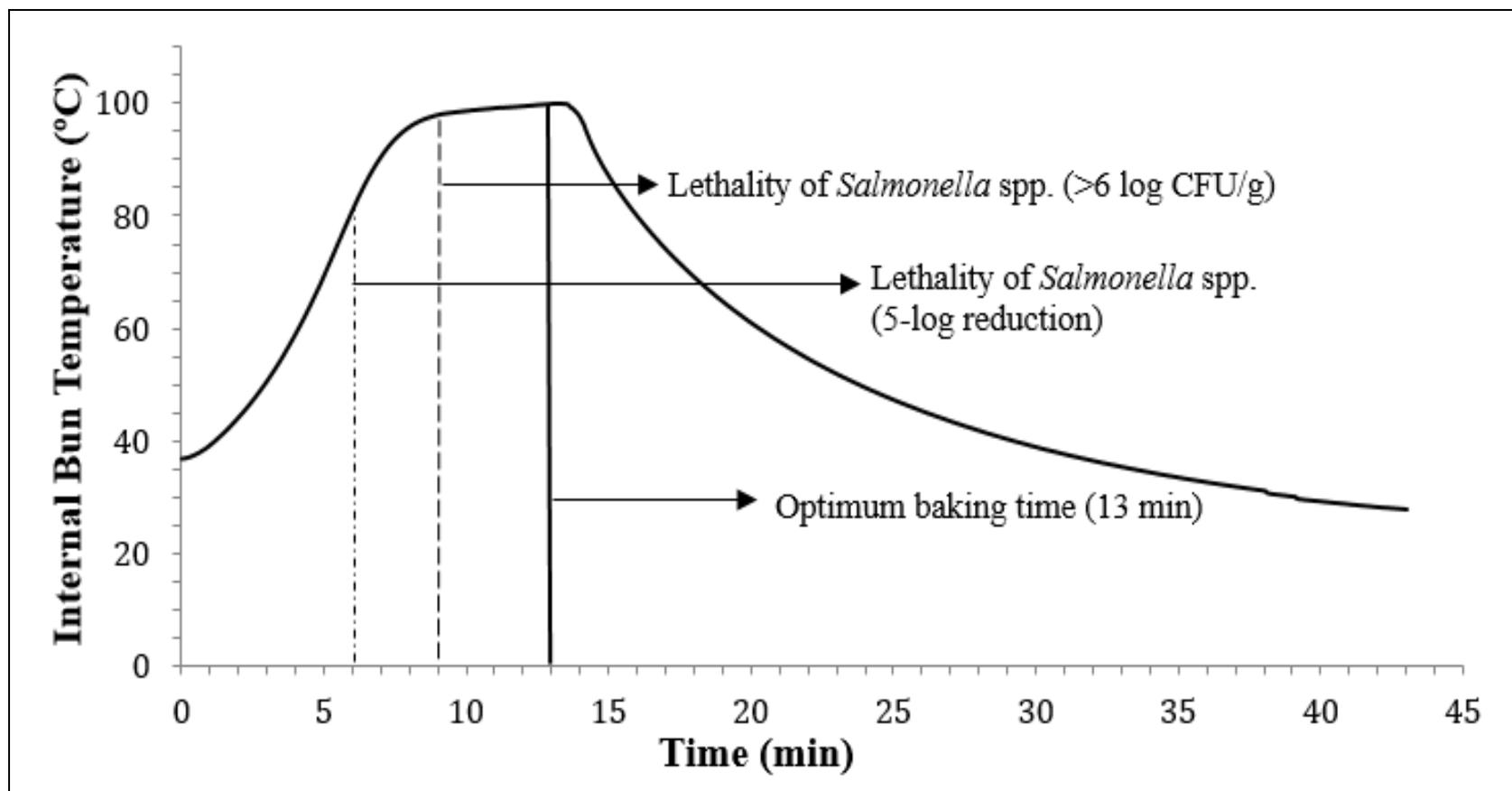


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Internal Temperature Data



Results

- ❑ No viable *Salmonella* serovar cells were enumerated by direct plating on selective or injury-recovery medium (detection limit of 0.22 log CFU/g), or after selective enrichment after the minimum 9 min of baking
- ❑ *E. faecium* on both selective and injury-recovery media were observed up to 11 min of baking
- ❑ No viable yeast cells were detected after the 9 min baking point
- ❑ It is likely that the yeast were actually inactivated to undetectable levels at shorter baking times.



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Results (cont)

- ❑ The crumb temperatures increased to $\sim 100^{\circ}\text{C}$ during the first 8 minutes of baking, and remained at this temperature for the next 6 min
- ❑ The greater D- and Z-values of *Salmonella* spp. and *E. faecium* as determined on the injury-recovery media confirms that a sub-population of injured bacterial cells is able to survive heating in dough at the three temperatures studied
- ❑ D and Z values of *S. cerevisiae* were much lower compared to those of *Salmonella* spp. and *E. faecium*



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Conclusions

- ❑ The data clearly demonstrates that the typical hamburger bun baking process utilizing oven temperatures $\geq 218.3^{\circ}\text{C}$ (425°F) is extremely efficient in reducing very high levels of *Salmonella* spp. (>6 log CFU/g) for all baking times
- ❑ For the first time there is a scientific proof that a normal baking process can reduce *Salmonella* population by > 6 log cfu/g.
- ❑ *E. faecium* demonstrated greater thermal resistance compared to *Salmonella* spp. and *S. cerevisiae*
- ❑ Considering the internal temperature vs. time recorded in this study, it is clear that all the *Salmonella* cells were destroyed within 9 min, prior to the optimum bake time (as determined and utilized in these studies).



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Conclusions (cont)

- ❑ The low thermal tolerance of *S. cerevisiae* relative to *Salmonella* limits its usefulness as a potential surrogate for process validations
- ❑ Greater survival of *E. faecium* during bun baking and higher D-values of *E. faecium* compared to that of *Salmonella* spp. suggest that *E. faecium* can be used as a surrogate for *Salmonella* spp. for baking studies in processing facilities, if needed
- ❑ Thermal resistance ranking:
E. faecium* > *Salmonella* serovars > *S. cerevisiae
- ❑ It is necessary and valuable to conduct additional research involving multiple baked goods categories to demonstrate similar effects reported in this study



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Manuscript Publication



Baking Validation for Hamburger Buns

1 **Validation of Baking to Control *Salmonella* Serovars in Hamburger Bun**
2 **Manufacturing, and Evaluation of *Enterococcus faecium* ATCC 8469 and**
3 ***Saccharomyces cerevisiae* as Nonpathogenic Surrogates for Thermal Process**
4 **Validation**

5
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11 **Keywords:** baking, *Salmonella*, *Enterococcus faecium*, yeast, D-value

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Highlights from July 20th, 2015 FDA Meeting:

Venue: FDA headquarters, Washington D.C., USA.

- ❑ A first study presented to the FDA that indicated a proactive, industry approach to comply FSMA - The preventive controls for human foods (PCHF) rule
- ❑ FDA had positive comments on AIB' s intent to design kill-step validation protocol for bakery products and provide on-site support, and training for US baking industries
- ❑ Overall, the FDA liked the kill-step validation research done so far, and thought the bakery products selected for the next phase were appropriate, and
- ❑ Interaction with the FDA will continue as we design and carry out next generation bakery product validation studies

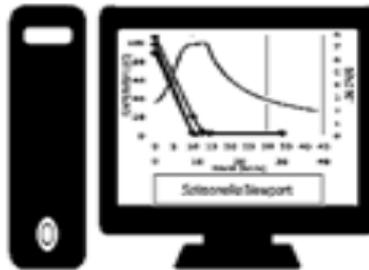


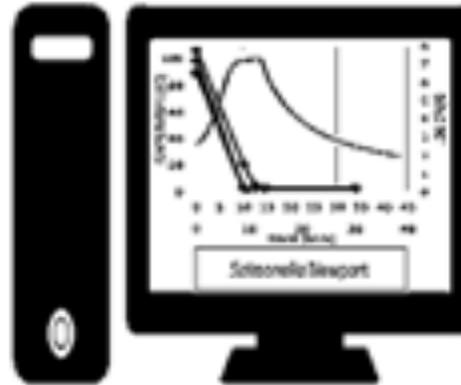
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- **Objective:**
- The BPKC is designed to assist bakers in evaluating the lethality of their process for destruction of *Salmonella* spp. in a variety of bakery products





- ❑ A microbial kinetics based interactive spreadsheet/calculator
 - ❑ Based on internal temperature and time parameters

Simple Procedure

- Download the BPKC to your computer from AIB's website
- Download the temperature profile (time-temperature data) from a data logger to an Excel sheet
- Format the temperature profile (time-temperature data), such that the time is in intervals of one minute and the temperature is in °F
- Enter at least 20 time-temperature data points to BPKC to accurately calculate the lethality of the process

- NOTE: Format the time such that you include at least the temperature data from 120°F during heating to the same temperature while cooling the product. It is acceptable to change the time intervals to larger values (such as ≥ 2 min) in case the process is longer and the product is at temperature 120°F for longer periods of time



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Microbial Kinetics: Terminologies

- **D-Value:** This indicates time in minutes at a constant temperature, that is necessary to destroy 90% or 1 log of the organism present at a given reference temperature.
- **z-Value:** This is the temperature increase required to reduce the thermal death time by a factor of 10.
- The z-value is constant for a given microorganism strain in a given product.
- **F-Value:** This is the process lethality or the time in minutes, at a specific temperature required to destroy a certain number of viable cells.



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D value,
Z value and
T-ref

Time (min)	Core Temp (°F)
0	63
4	66
10	76
15	83
19	100
22	112
25	118
31	126
37	131
40	133
43	135
46	137
52	145
58	147
61	149
64	148
67	146
73	139
76	120
82	112
84	105



Process
lethality
in terms
of log
reductions

D, Z and T-ref
are obtained
from AIB's KSV
study

Time x Temp data
obtained from the
MOLE® data
logger



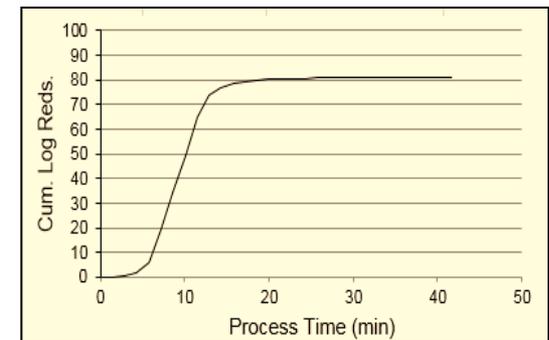
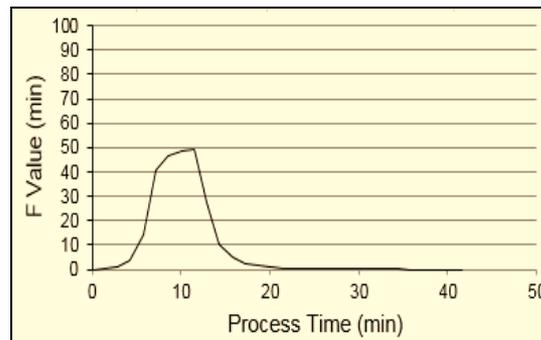
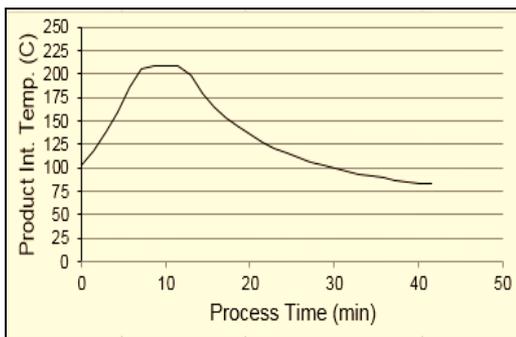
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BPKC: Output

- ❑ Determines F - value and cumulative F - value
- ❑ Automatically determines the total process lethality (e.g., 5 log) for *Salmonella*
- ❑ Generates three graphs: Product internal temperature, F value/min and cumulative log reductions



Benefits of BPKC:

- If the desired log reduction is achieved for the baking process and pathogen of concern, the process lethality report generated can be used as guidance and as a supporting documentation for
- **FSMA validation and verification process**



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Additional Research is Important Because

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- ❑ There are more than 2,000 bakery products in the market.
- ❑ Bacteria respond/behaves differently at varying moisture levels, pH, fat content etc. e.g., *Salmonella* has shown the highest resistance in low water activity and high fat foods.
- ❑ Hence, it is necessary and valuable to conduct additional research involving multiple baked goods categories with varied moisture contents, pH, water activities (aw) etc.
- ❑ Also, it is important to publish the next generation baking validation research findings, thus providing documentary evidence for the benefit of bakery industry's food safety needs.



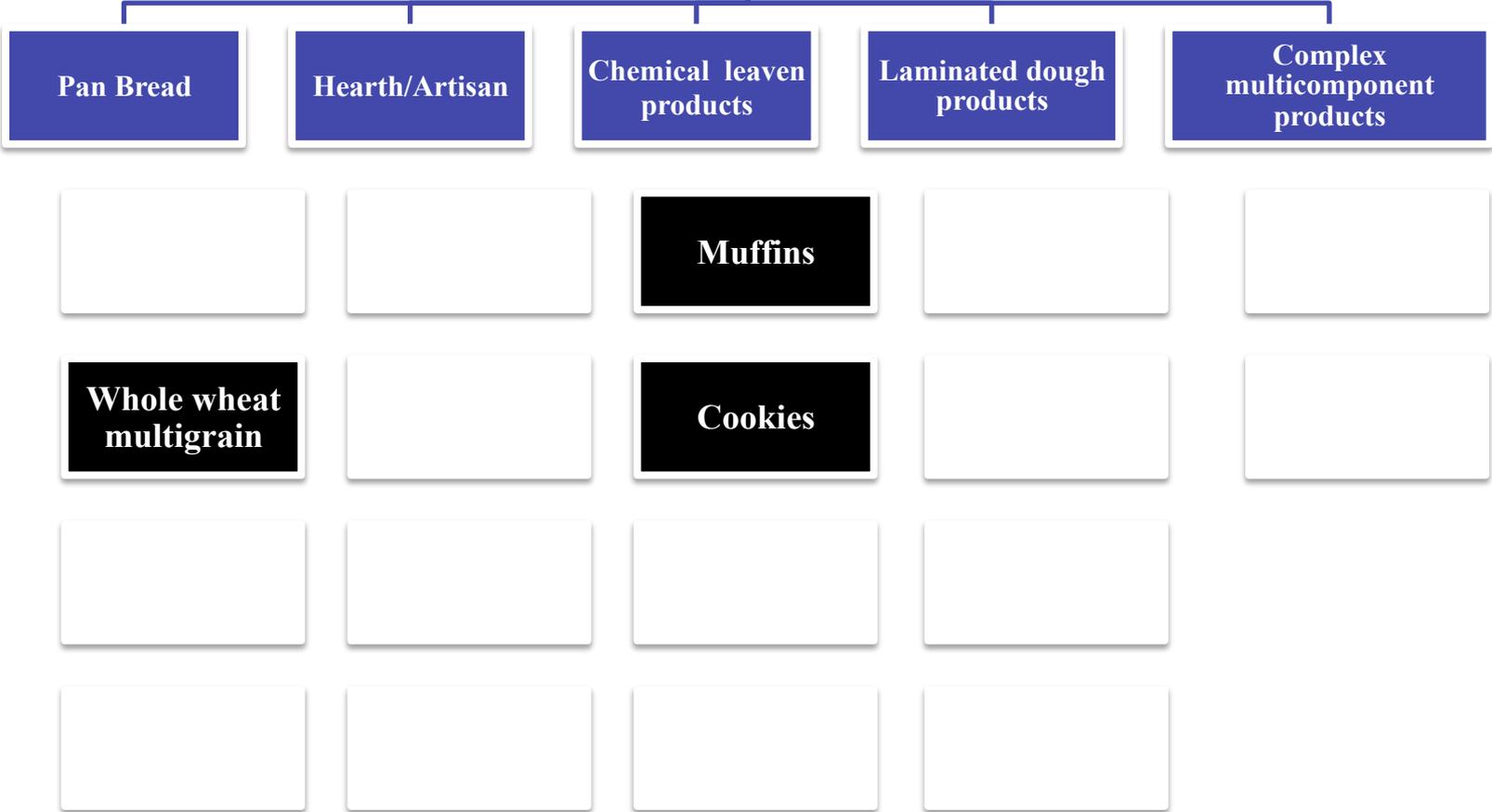
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KSV Gen- 2 Validation Research:

Major Bakery Products Category



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Baking Validation Next Generation

Research: *Criteria and Justification*



Whole grain wheat bread

- Size (1lb loaf)
- High moisture
- High water activity (a_w)



Muffins

- Chemically leavened
- High sugar
- High moisture
- High fat



Cookies

- Chemically leavened
- Multi-component
- Low moisture
- Low water activity (a_w)

KSV Gen 2: Research Objectives

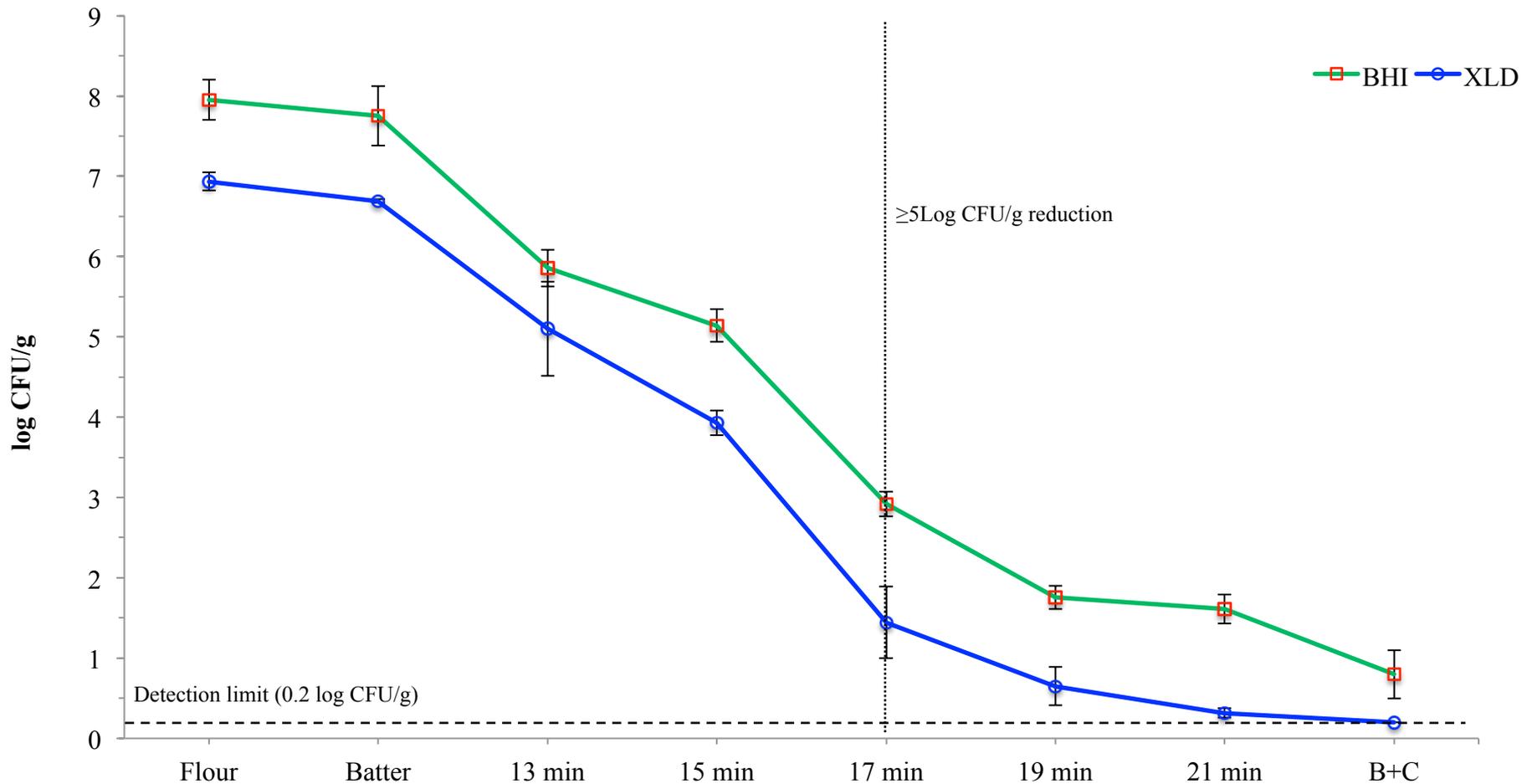
- Validate the baking process as a kill step in reducing *Salmonella* cocktail (*S. Typhimurium*, *S. Newport*, and *S. Senftenberg*), and
- Determine the heat resistance pattern viz, D-value, and Z-value of *Salmonella* spp. during the baking process



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Salmonella spp. (3-strain cocktail) counts of muffins during 21 min of baking at 375°F (190.6°C) and 30 min of ambient air cooling (B+C).

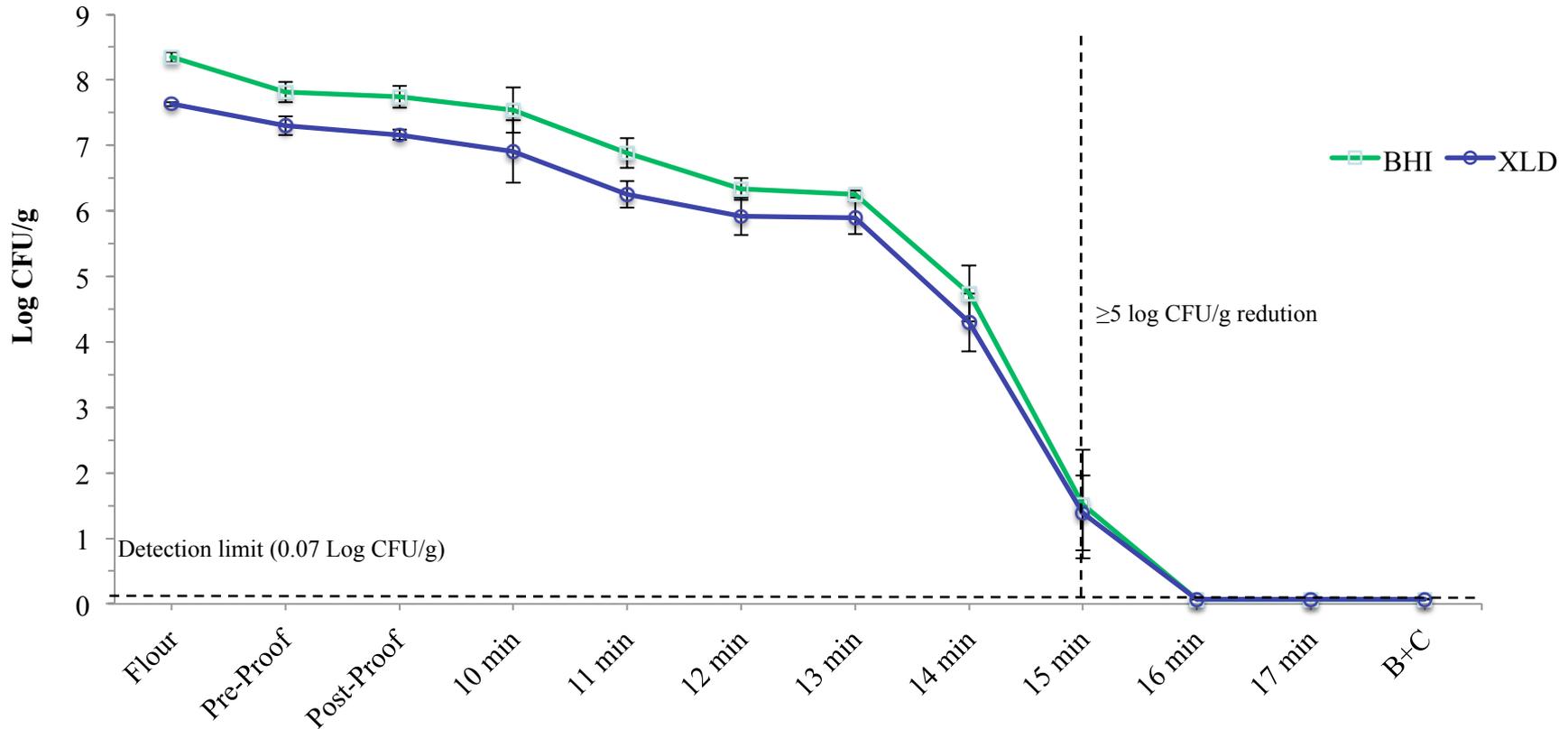


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Salmonella Population in Bread during Baking



Salmonella spp. counts (7-strain cocktail) in bread during 35 min of baking at 375°F (190.6°C) and 60 min of ambient air cooling (B+C).



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Short and long term benefits of this research to the baking industry

- Pathogen free bakery products, assuring greater safety possible
- Protects consumers, builds confidence
- Helps in determining an effective treatment option
- Demonstrates compliance to the FDA-FSMA act.
- Can help bakery industries avoid repetition and duplication use of resources for kill-step validation research, and
- Offers a pathogen inoculation option for companies with limited resources and capabilities.



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Thank You



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