Survival and thermal resistance of *Salmonella* in dry and hydrated non-fat dry milk and whole milk powder during extended storage

Amninder Singh Sekhon
Graduate Research Assistant
School of Food Science
Washington State University

Background

- Hometown – **Ferozepur, Punjab**
- B. Tech. in **Dairy Technology** – Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India (2018)
- M.S. in **Food Science** – Washington State University (2020)
- Ph.D. in **Food Science** – Washington State University (2023)

Why *Salmonella*?

- Ability to survive in harsh conditions
- In the U.S., 1.35 million illnesses with 26,500 hospitalizations and 450 deaths annually (CDC, 2020)
- Infants, elderly and immuno-compromised individuals are at high risk
- The infectious dose for *Salmonella* infection is <1,000 cells, but can be as low as 1 cell
- *Salmonella* is responsible for the greatest number of bacterial foodborne infections in the U.S.

Concern in milk powders

- Used as an ingredient in many foods, especially infant formula
- The incidences of salmonellosis among infants are higher compared to other age groups
- Post pasteurization contamination of milk can occur
- Presence of *Salmonella*, even in low numbers in low water activity (a_w) foods such as milk powders can pose a significant health risk

Research objectives

- To determine the survivability of *Salmonella* in dry and hydrated nonfat dry milk (NFDM) and whole milk powder (WMP) during 180 days of storage
- To determine the effect of storage and powder type (NFDM and WMP) on the thermal resistance (D- and z-values) of *Salmonella*

Experimental Design

- This study was designed as a two factorial (storage day and powder type) randomized complete block design, with three replications as blocks
- The linear regression graphs for calculating D- and *z*-values were plotted using Microsoft Excel 2020
- The D- and z-values of *Salmonella* at specific temperatures within dry or hydrated milk powders during the storage period were compared at $P \leq 0.05$ using two-way ANOVA and Tukey's Test

D- and z-values

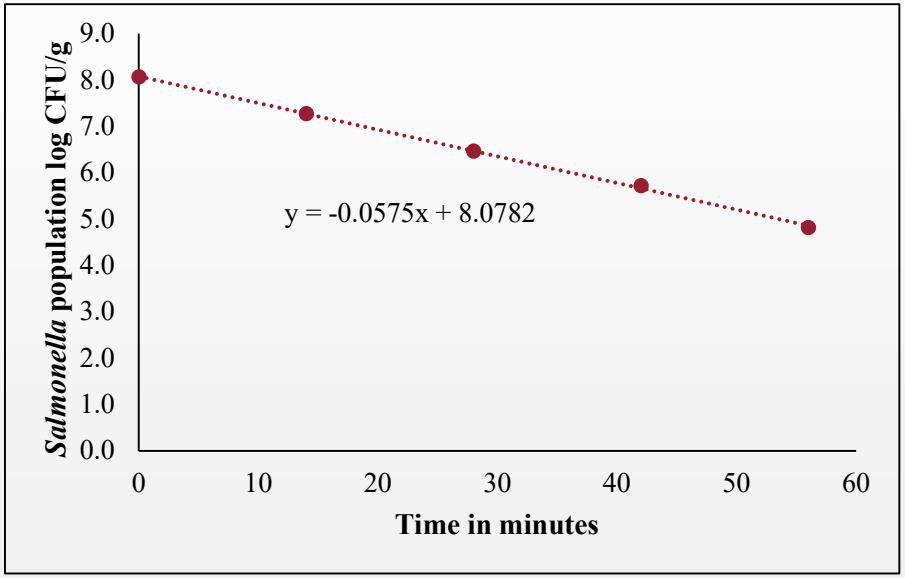


Figure1. Log *Salmonella* population versus time used to calculate D-values

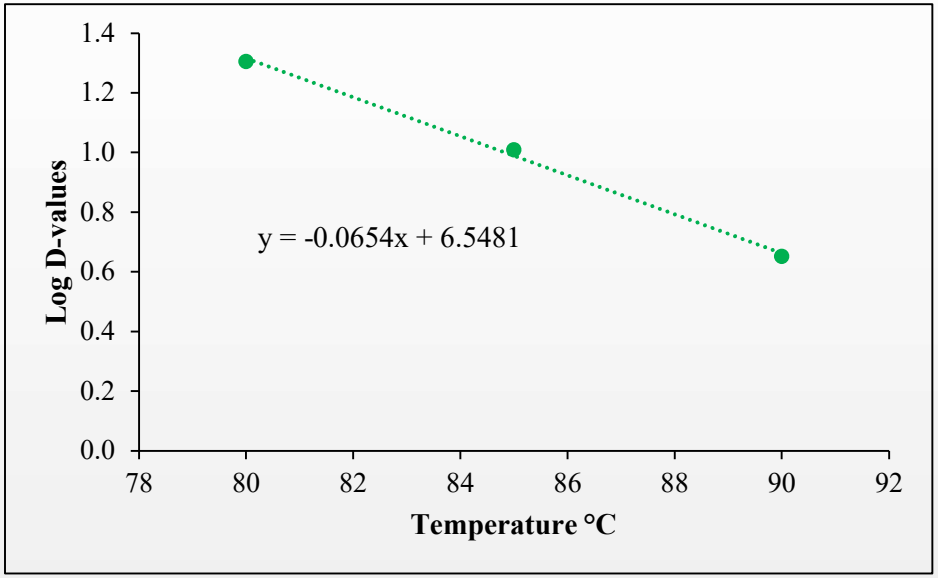


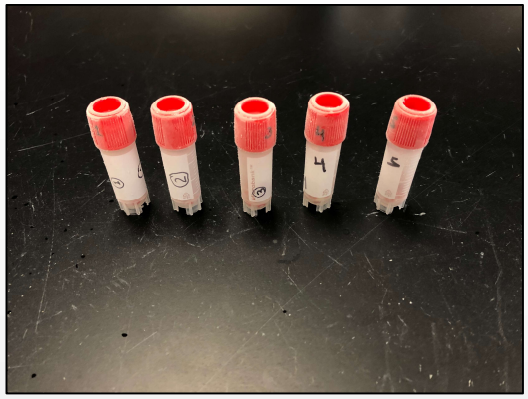
Figure2. Log D- values of *Salmonella* versus temperature used to calculate z-values

Materials and methods

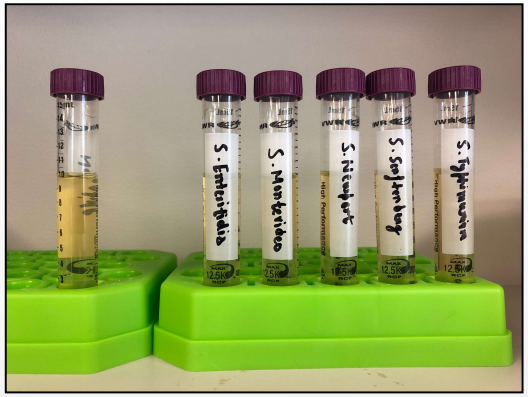
***Salmonella enterica* subsp. *enterica* serovars used in the study:**

- Enteritidis (ATCC® BAA-708)
- Montevideo (ATCC® BAA-710)
- Newport (ATCC® 6962)
- Senftenberg 775W (ATCC® 43845)
- Typhimurium (ATCC® 14028)

Salmonella culture propagation and inoculum preparation

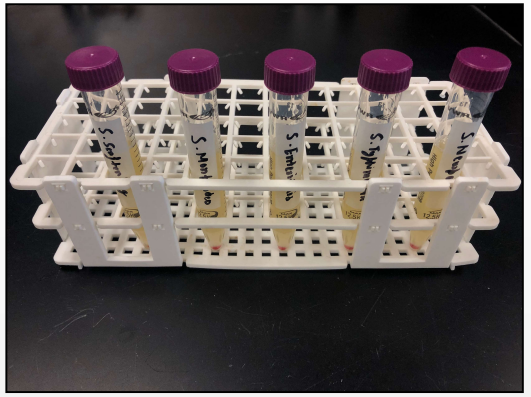


Frozen beads



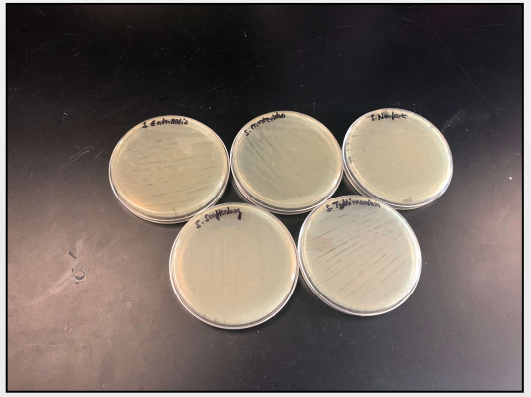
10 mL BHI broth

37°C for 24 hr



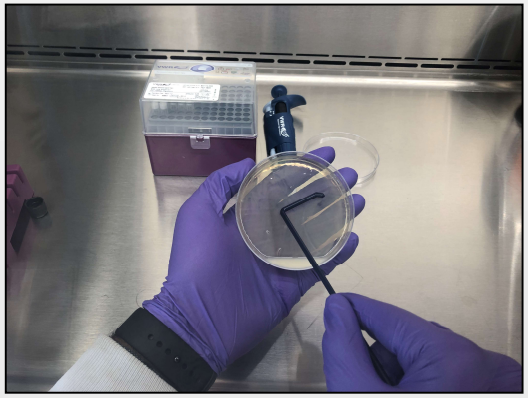
Propagated cultures

37°C for24 hr

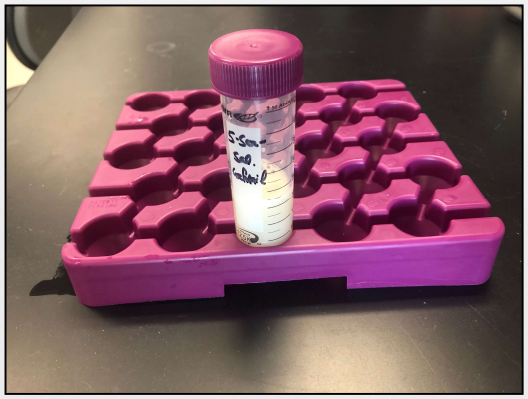


Bacterial lawns

37°C for 24 hr



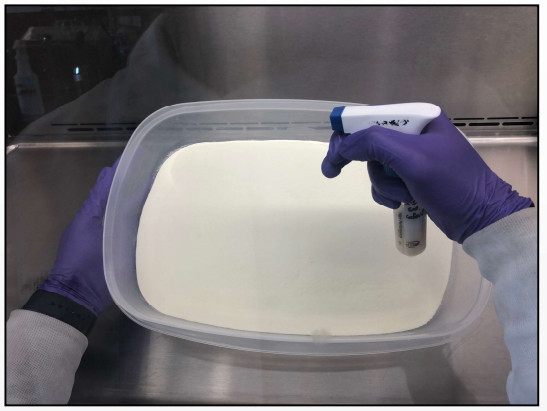
Harvesting the lawns



Master inoculum

Inoculation of milk powders

- Milk powders were mist inoculated and dried back to original pre-inoculation weight to achieve ~8 log CFU/g population
- Inoculated milk powders were mixed thoroughly, transferred to Ziplock bags, stored in sealed airtight tubs at ambient temperature (~20°C) for 180 days, and analyzed at regular intervals



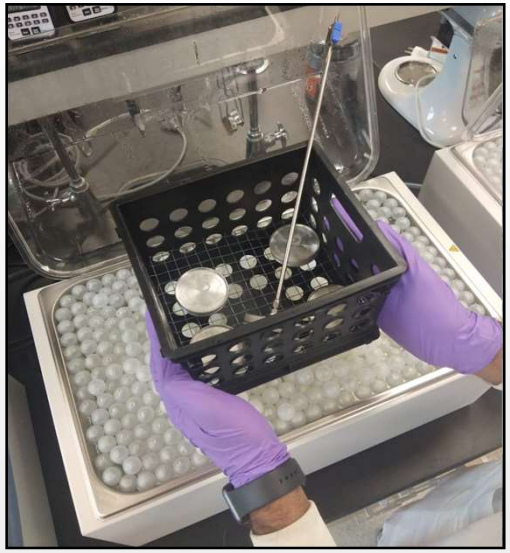
Mist inoculation of NFDM

Drying of NFDM

Thermal inactivation parameters

- Inoculation day was considered as day 0
- D- and z-values were determined every 30th day starting from day 1 until day 180
- For determining D- and z-values of *Salmonella* in hydrated milk powders, the hydration [13 % (w/v) total solids] was performed just before the heat treatments
- Surviving *Salmonella* population was enumerated using **injury recovery media** i.e., brain heart infusion (BHI) agar overlaid with XLD agar

Thermal treatments



Thermal-death-time (TDT) discs and hot water bath used for heat treatments

Ice water bath for rapidly cooling samples in TDT disks

Thermocouples used for monitoring product and water temperature

Heat treatments

Table 1. Sampling times used for calculating D-values of 5-serovar *Salmonella* cocktail in nonfat dry milk (NFDM) and whole milk powder (WMP) at respective temperatures

	D-value Temp. (°C)	Sampling time (minutes)
NFDM and WMP	80	56
	85	28
	90	14
Hydrated NFDM and WMP	59	12
	62	6
	65	2

Table 2. *P*-values of the main effects and interactions of the main effects for various parameters of dry and hydrated milk powders evaluated during the storage of 180 days

	P-value		
Parameters	**Milk powder type**	**Storage day**	**Milk powder type × storage day**
Salmonella survival in dry powders	0.051	<0.001*	0.350
Salmonella survival in hydrated milk	0.104	<0.001*	0.302
D- value of dry powders at 80°C	<0.001*	<0.001*	<0.001*
D- value of dry powders at 85°C	<0.001*	<0.001*	0.021*
D-value of dry powders at 90°C	<0.001*	0.005*	0.034*
D-value of hydrated milk at 59°C	0.181	0.206	0.077
D-value at of hydrated milk 62°C	0.911	0.159	0.594
D-value at of hydrated milk 65°C	<0.001*	0.231	0.904
z- value in dry powders	0.059	0.546	0.566
z- value in hydrated milk	0.059	0.991	0.675
Water activity of dry powders	<0.001*	<0.001*	0.083*
pH of dry powders	<0.001*	<0.001*	<0.001*
pH of hydrated milk	0.273	0.487	0.262

* Main and/or interaction of main effects were significant ($P \leq 0.05$)

Water activity (a_w) of dry milk powders

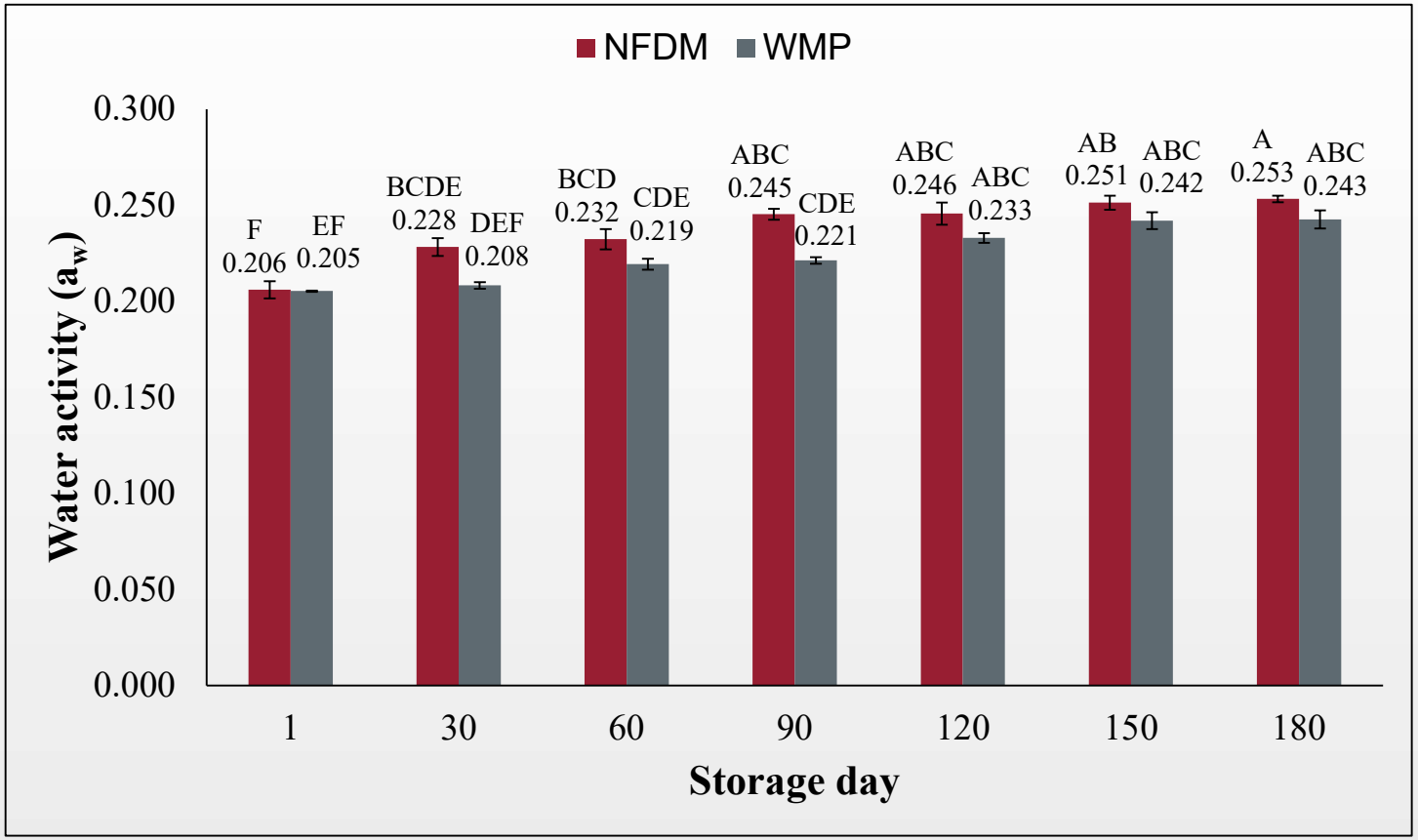


Figure 1. Water activity (a_w) of nonfat dry milk (NFDM) and whole milk powder (WMP) during storage as a function of storage day × powder type

pH of hydrated milk powders

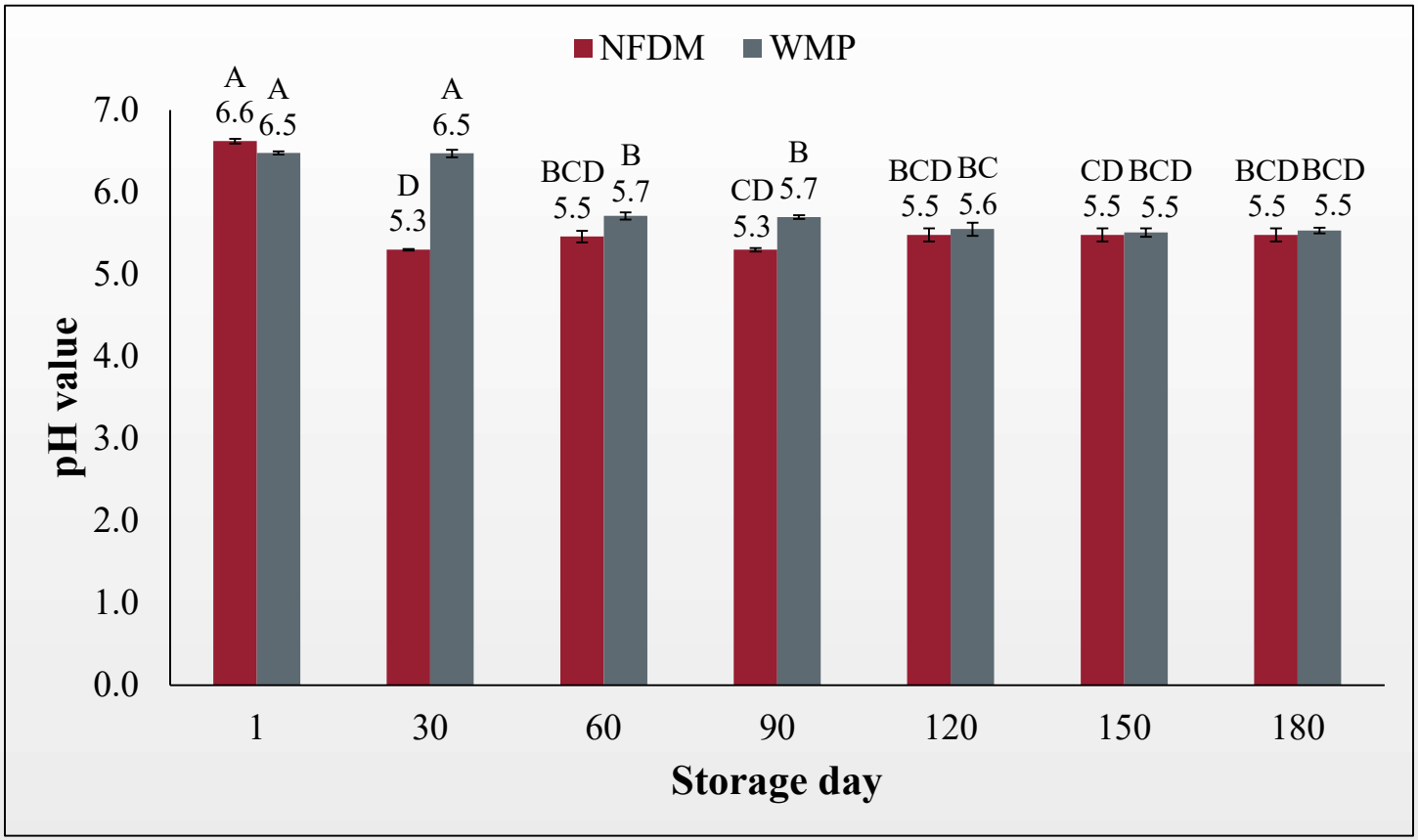


Figure 2. pH of nonfat dry milk (NFDM) and whole milk powder (WMP) during storage as a function of storage day × powder type

Salmonella survival in dry powders

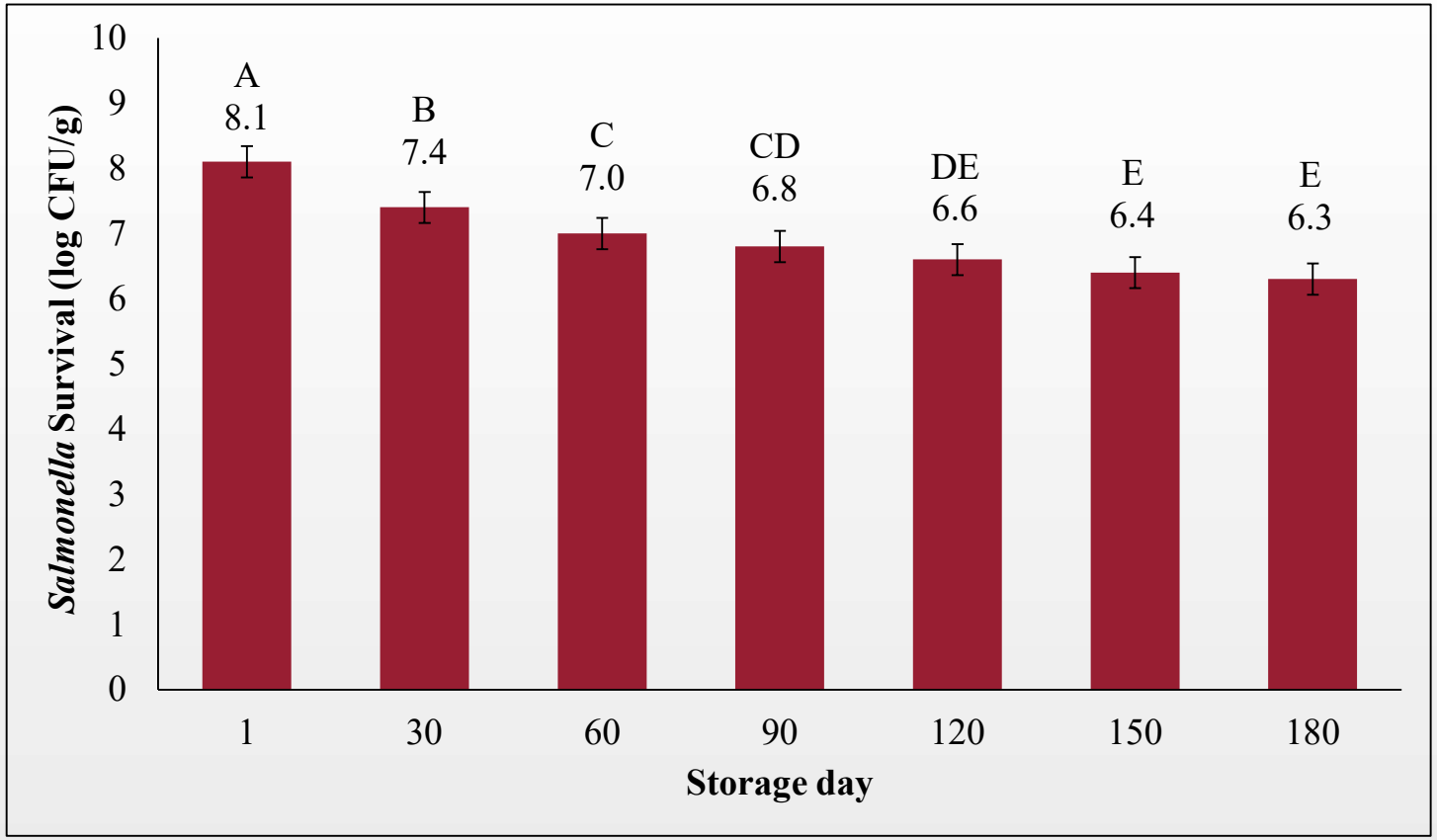


Figure 3. Population of 5-serovar *Salmonella* cocktail (log CFU/g) during storage as a function of storage day in both dry nonfat dry milk (NFDM) and whole milk powder (WMP)

Salmonella population in hydrated milk powders

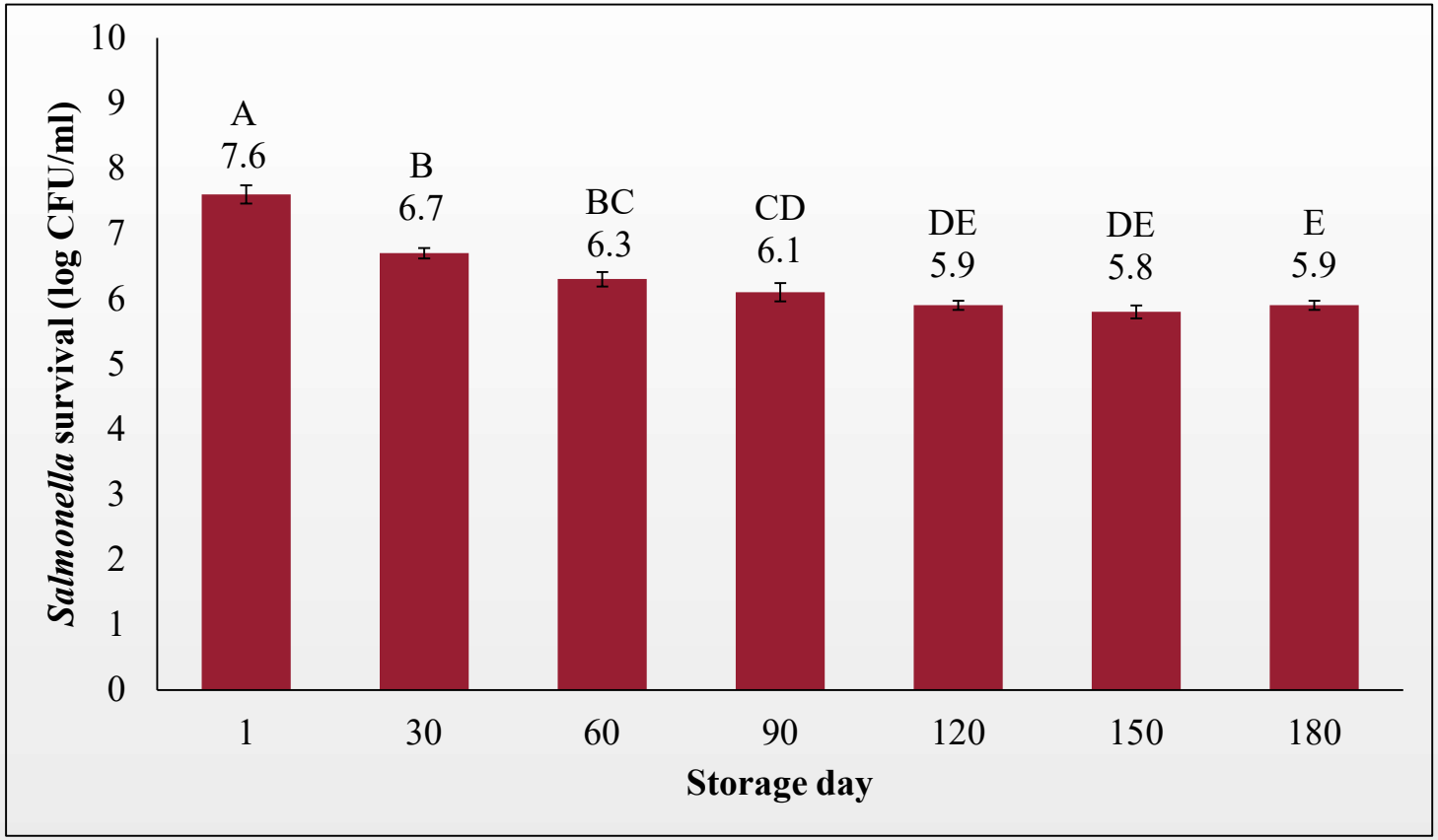


Figure 4. Population of 5-serovar *Salmonella* cocktail (log CFU/ml) during storage as a function of storage day in both hydrated nonfat dry milk (NFDM) and hydrated whole milk powder (WMP)

Salmonella D-values in milk powders (80°C)

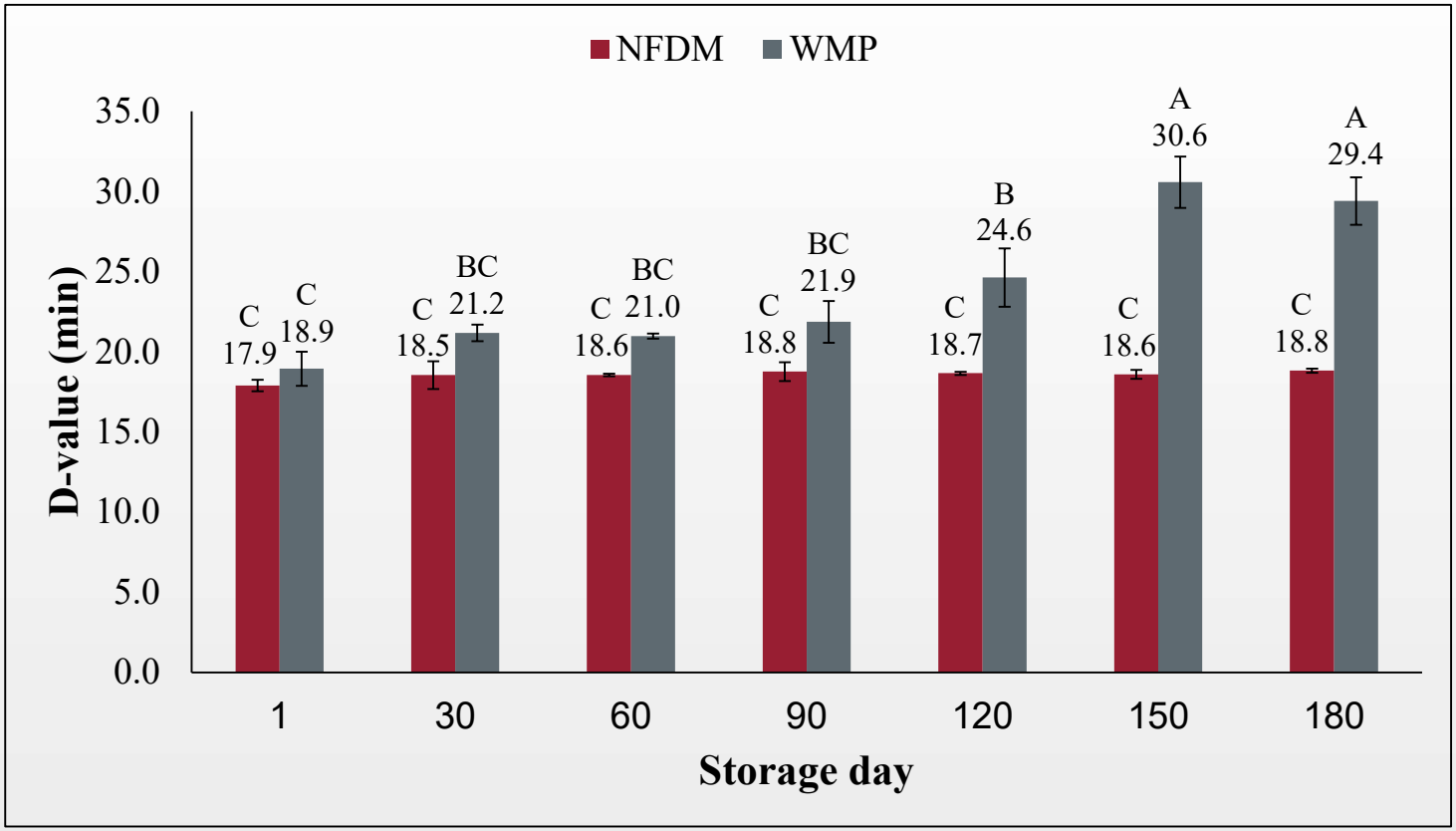


Figure 5. D-values of 5-serovar *Salmonella* cocktail in nonfat dry milk (NFDM) and whole milk powder (WMP) during storage as a function of storage day × powder type at 80°C

Salmonella D-values in milk powders (85°C)

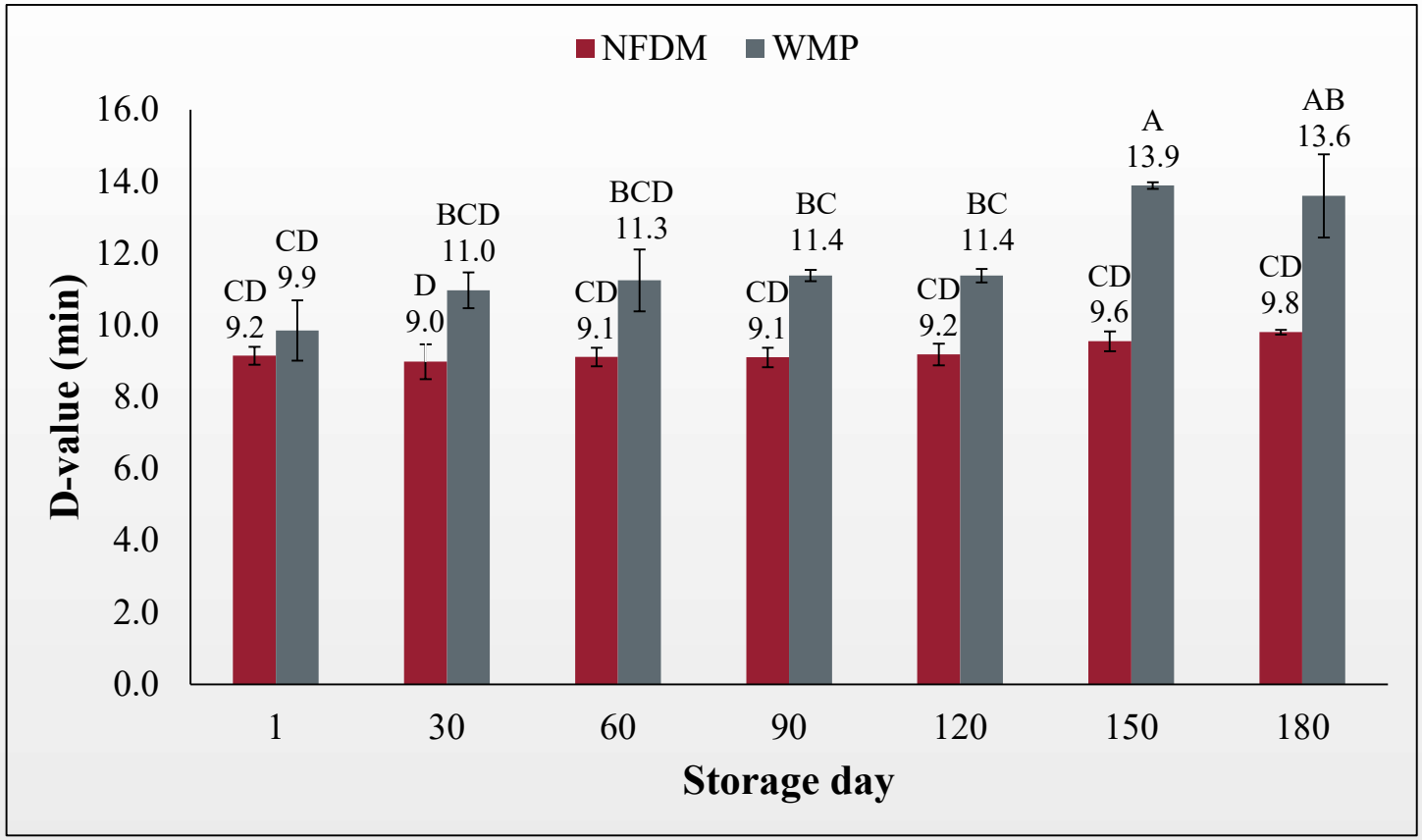


Figure 6. D-values of 5-serovar *Salmonella* cocktail in nonfat dry milk (NFDM) and whole milk powder (WMP) during storage as a function of storage day × powder type at 85°C

Salmonella D-values in milk powders (90°C)

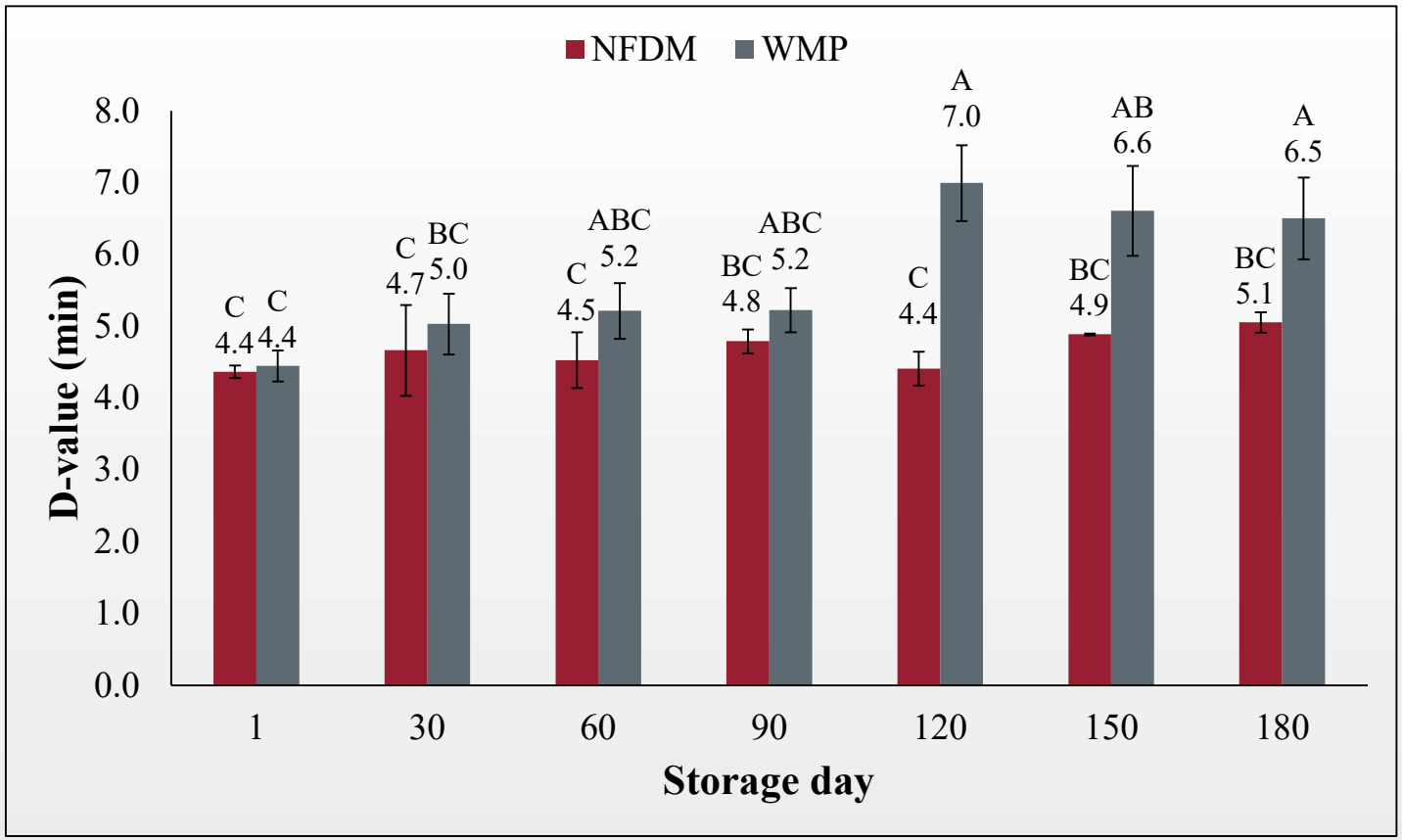


Figure 7. D-values of 5-serovar *Salmonella* cocktail in nonfat dry milk (NFDM) and whole milk powder (WMP) during storage as a function of storage day × powder type at 90°C

No significant main or interaction effects

Table 3. Mean* parameters values of dry or hydrated nonfat dry milk (NFDM) and whole milk powder (WMP) with no significant main effects and interaction of main effects.

Dry milk powders	**Hydrated milk powders**			
z-value (°C)	**z-value (°C)**	***Salmonella* D-value at 59°C (min)**	***Salmonella* D-value at 62°C (min)**	**pH**
16.3 ± 0.57	6.4 ± 0.20	6.2 ± 0.12	2.5 ± 0.15	6.5 ± 0.03

* Values are mean (±SE; n=42) of parameters for all storage days and both hydrated milk powders

Summary

- *Salmonella* population was reduced by 1.8 log CFU/g in milk powders and 1.7 log CFU/ml in corresponding hydrated milk
- The thermal resistance of *Salmonella* in NFDM during 180 days of storage stayed similar
- However, during 180 days of storage, thermal resistance of *Salmonella* in WMP increased by 1.4-1.6-fold
- The data from this study can help develop accurate prediction models for the survival and the heat resistance of *Salmonella* during the extended storage of milk powders

Acknowledgments

- Dr. Minto Michael (Major advisor)
- Lab mates:
- Arshdeep Singh
- Phoebe Unger
- Yaeseol Yang
- Monipel Babb

THANK YOU