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Pathogen control in cheese brines using hydrogen peroxide

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Background

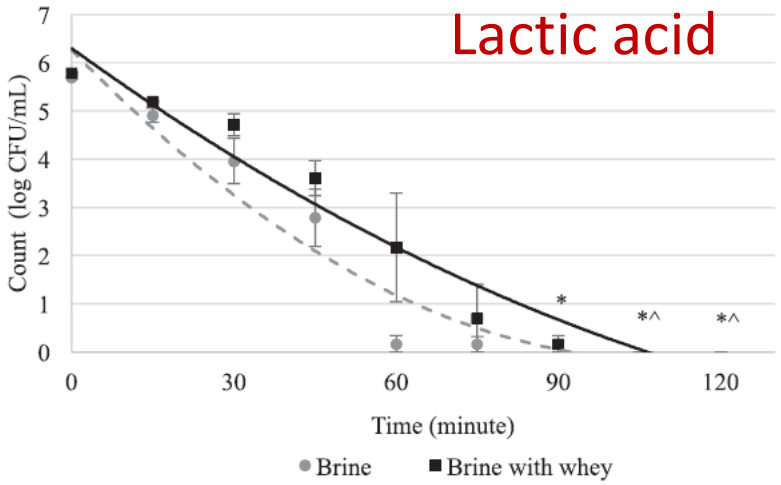
- Brining/salting important step during cheese manufacturing
  - Pure salt solutions: correlation between salt and water activity
- Brine can serve as a reservoir of salt tolerant pathogens
  - *Listeria monocytogenes*
    - "zero tolerance" pathogen
    - Growth at 13% salt; survive in up to 30% salt; lower limit for growth a_w 0.92
  - *Staphylococcus aureus*
    - Requires growth in high levels to develop enterotoxin
    - Poor competitor
    - Salt growth limits affected by pH
    - Tolerance 20% salt; lower limit for growth a_w 0.86

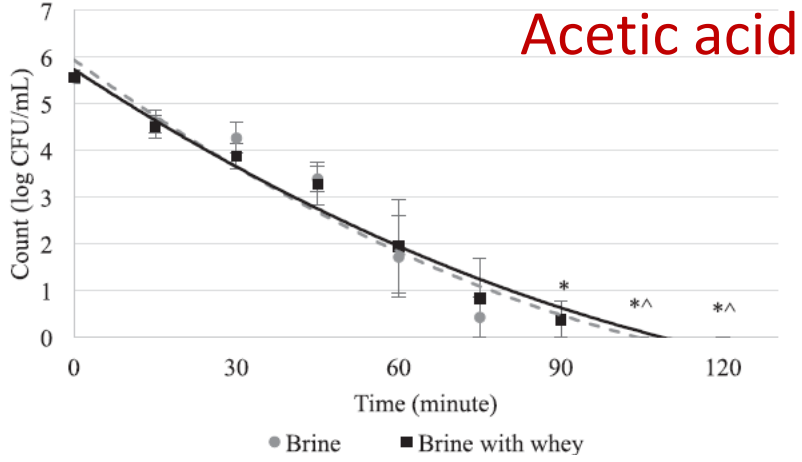
% NaCl	Aw
0.9	0.995
1.7	0.99
3.5	0.98
7.0	0.96
10.0	0.94
13.0	0.92
16.0	0.90
22.0	0.86

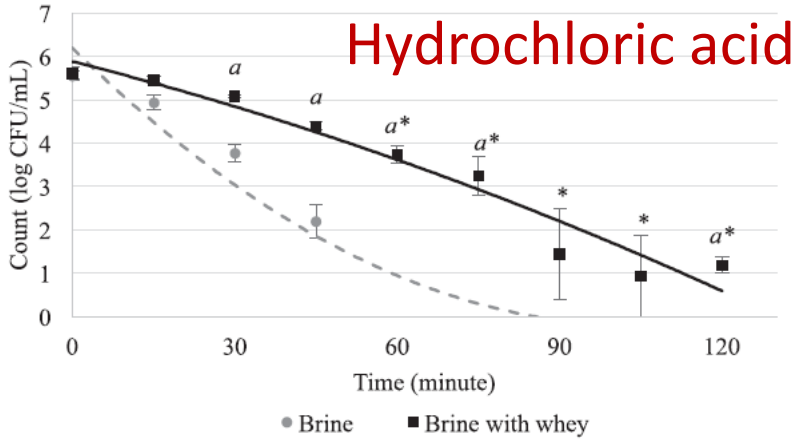
Mitigating Strategies for Brine Safety

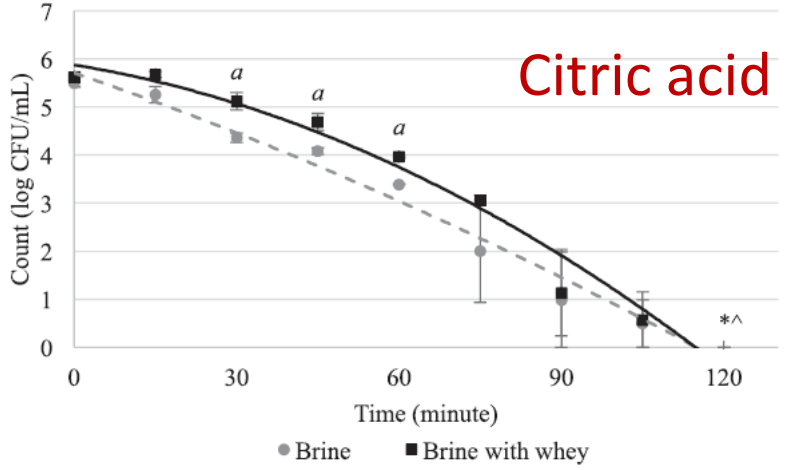
- Robust brine management program with effective sanitation and GMPs during the brining process to prevent cross-contamination
  - Environmental monitoring program
    - Zone 1 (Product Contact Surfaces) – Indicator organisms (yeast & molds; Enterobacteriaceae; coliforms
    - Zones 2 & 3 (Non-product Contact Surfaces) – *Listeria* spp.
  - Monitor salinity, pH, temperature and % solids of the brine
- Physical brine skimming, filtration, lethal antimicrobial

Acidification of model cheese brines (pH 2.0)



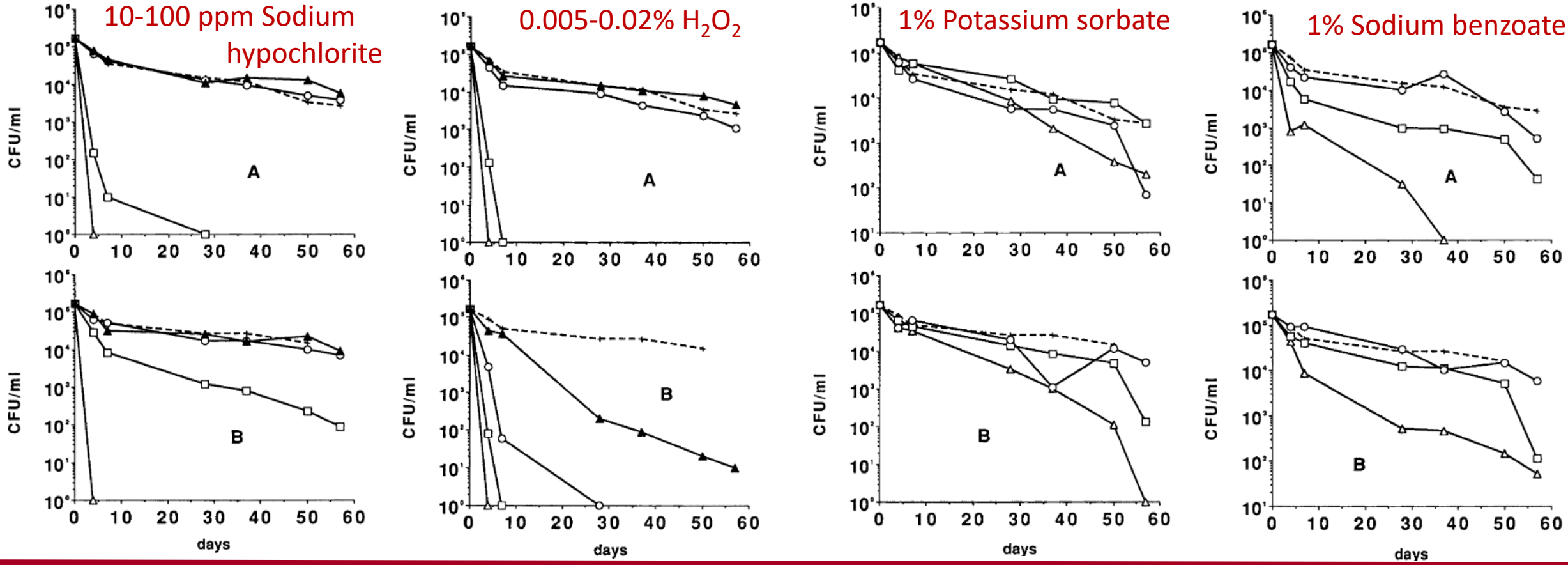






Brown, Stephanie RB, et al. "Acidification of model cheese brines to control *Listeria monocytogenes*." *Journal of food protection* 81.1 (2018): 79-83.

Chemical treatments that have reduced the survival of *L. monocytogenes* in commercial cheese brines



Larson, A.E., Johnson, E.A., Nelson, J.H., 1999. Survival of *Listeria monocytogenes* in Commercial Cheese Brines. Journal of Dairy Science 82, 1860–1868.. https://doi.org/10.3168/jds.s0022-0302(99)75419-6

Objective

Determine the interactive effect of pH, salt, hydrogen peroxide (H_2O_2), temperature and background microbial populations on the inactivation of pathogens in cheese brine.

Phase 1: Inactivation of *L. monocytogenes* in Fresh Model Brines

Hydrogen peroxide	• 0 ppm • 50 ppm • 100 ppm
Model brines (filtered)	• 10% salt pH 4.6 • 10% salt pH 5.4 • 20% salt pH 4.6 • 20% salt pH 5.4
Temperatures	• 10°C (50°F) • 15.6°C (60°F)

- Inoculated with 5.5-log CFU/mL of 5 – strain cocktail of acid adapted *L. monocytogenes*
  - LM301 (Cheddar Isolate,1/2a)
  - LM108M (low moisture, low pH salami isolate, 1/2b)
  - LM310 (Feta cheese isolate, 4b)
  - FSL-R2-500 (Hispanic style soft cheese isolate, 4b)
  - FSL J1-110 (Jalisco cheese isolate, serotype 4b)
- Assayed at t=0, 24 h and 7 days (1 week)


10% salt, pH 4.6
10% salt, pH 5.4
20% salt, pH 4.6
20% salt, pH 5.4
Model Brine, 10°C, 0 ppm H2O2
Model Brine, 10°C, 50 ppm H2O2
Model Brine, 10°C, 100ppm H2O2
Model Brine, 15.6°C, 0 ppm H2O2
Model Brine, 15.6°C, 50 ppm H2O2
Model Brine, 15.6°C, 100ppm H2O2
Average log change in populations of L. monocytogenes (log CFU/ml)
0
-1
-2
-3
-4
-5
-6
1 hour
24 hour
1 week

Summary: Phase 1

- No growth of *L. monocytogenes* in any brines
  - pH 4.6-5.4; 10-20% NaCl
- Inactivation of *L. monocytogenes* was effective with 100 ppm of H_2O_2 (>4 log reduction) in all brines in a week.
- Factors that accelerate the inactivation of *L. monocytogenes* in cheese brines
  - Higher salt
  - Warmer brine temperatures

Objective – Phase 2

- To determine the effectiveness of hydrogen peroxide (H_2O_2) to reduce microbial loads in commercial cheese brines of varying cheese type, pH, and salt level while stored at different temperatures in one day and 1 week

Seven Commercial Brines

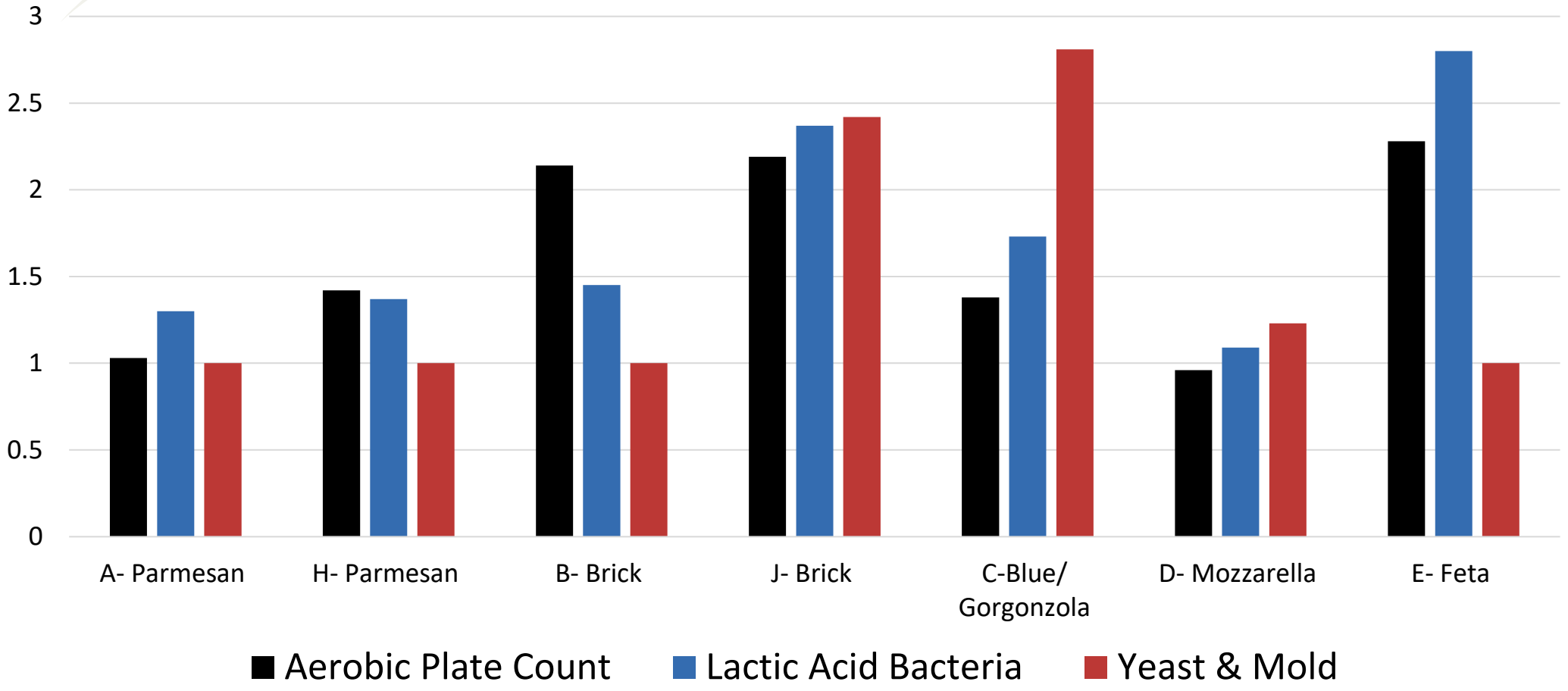
Designation	Cheese type	Use temperature	pH range	Salt range
A	Parmesan	50-53 F	5.00-5.10	21-22%
B	Brick	71-73 F	5.05-5.25	27-29%
C	Gorgonzola	50-60 F	4.55-4.75	20-23%
D	Mozzarella	30-32 F	5.30-5.40	25-28%
E	Feta	55 F	4.50-4.60	15-18%
H	Parmesan	53-55 F	5.05-5.20	27-29%
J	Brick	No data	5.40-5.55	10-19%

Experimental Design

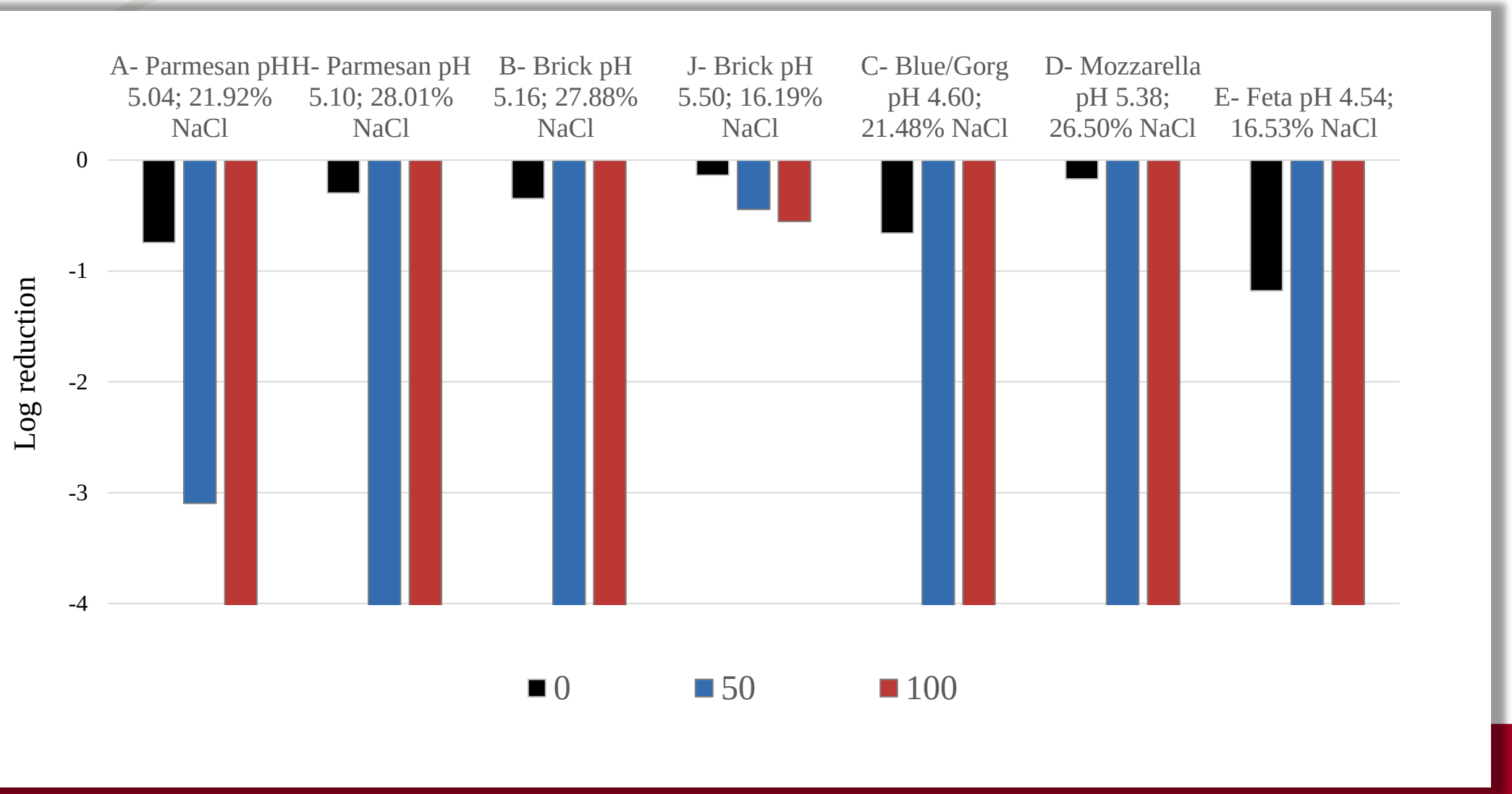
- 7 brines (see previous slide)
  - Each trial used brines from a different season (Summer/August 2020, Autumn/October 2020, Winter/January 2021)
- *L. monocytogenes* – acid adapted, 5-strain cocktail – 5.5-log CFU/mL
- *S. aureus* – acid adapted, 3-strain cocktail (FRI196E, whipped butter isolate, SEA; FRI S6, shrimp isolate, SEA&B; FRI 952, ham isolate, SEA&D) – 4.5-log CFU/mL
- H_2O_2 levels: 0 ppm, 50 ppm, 100 ppm
- Temperatures: 12.8˚C, 7.2˚C, 0˚C (only 12.8˚C for *S. aureus*)
- Testing at: Time 0, 1 day, 1 week
- Uninoculated samples tested for
  - Total aerobic plate count; Plate Count agar (PCA, 35C for 2 days)
  - Lactic acid bacteria; De Man, Rogosa, and Sharpe agar (MRS, 35C for 2 days, anaerobic)
  - Yeast/mold; acidified Potato Dextrose agar (PDA, 25C for 3-5 days)

Formulation	Typical brine temperature	Season	% Moisture	% Solids	pH	% NaCl	a_w
Parmesan A	10-11.7°C (50-53°F)	Summer	76.1	23.9	5.03	21.8	0.819
		Autumn	77.9	22.1	5.05	22.0	0.838
		Winter	77.1	22.9	5.05	21.9	0.825
			77.03±0.90	22.94±0.90	5.04±0.01	21.90±0.10	0.827±0.010
Parmesan H	11.7-12.8°C (53-55°F)	Summer	74.0	26.0	5.10	27.8	0.765
		Autumn	73.0	**27.0**	5.11	28.1	0.768
		Winter	73.3	26.7	5.10	28.15	0.761
			73.43±0.51	26.57±0.51	5.10±0.01	28.02±0.19	0.765±0.004
Brick B	21.7-22.8°C (71-73°F)	Summer	74.3	25.7	5.19	**28.6**	**0.766**
		Autumn	74.4	25.6	5.08	27.6	0.777
		Winter	74.6	25.4	5.20	27.5	0.775
			74.43±0.15	25.57±0.15	5.16±0.07	27.91±0.62	0.773±0.006
Brick J	4.4-7.2°C (40-45°F)	Summer	84.7	**15.3**	5.43	**12.5**	**0.909**
		Autumn	81.5	18.5	**5.54**	17.9	0.867
		Winter	81.2	18.8	**5.54**	18.2	0.869
			82.47±1.94	17.53±1.94	5.50±0.06	16.20±3.21	0.882±0.024
Blue/ Gorgonzola C	10-15.6°C (50-60°F)	Summer	76.5	23.5	4.59	20.76	0.827
		Autumn	76.5	23.5	4.60	22.14	0.822
		Winter	76.6	23.4	4.60	21.55	0.823
			76.53±0.06	23.47±0.06	4.60±0.01	51.48±0.69	0.824±0.003
Mozzarella D	-1.1-0°C (30-32°F)	Summer	76.7	23.3	5.40	25.89	0.797
		Autumn	75.4	24.6	5.34	26.99	0.789
		Winter	75.4	24.6	5.40	26.62	0.788
			75.83±0.075	24.17±0.75	5.38±0.03	26.50±0.56	0.791±0.005
Feta E	12.8°C (55°F)	Summer	78.7	21.3	**4.51**	16.04	0.872
		Autumn	78.9	21.1	4.56	15.63	0.874
		Winter	77.8	22.2	4.54	17.92	0.858
			78.47±0.59	21.53±0.59	4.54±0.03	16.53±1.22	0.868±0.009

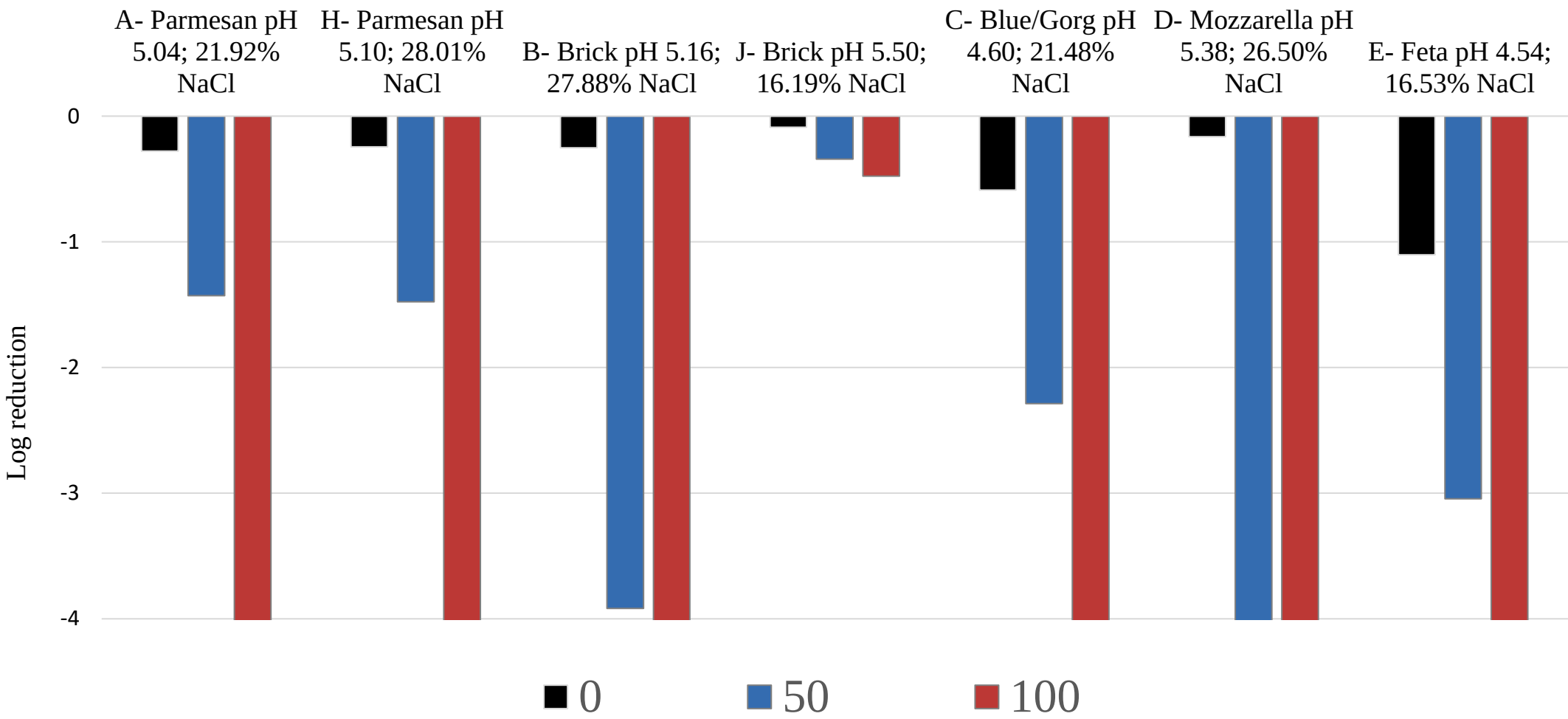
Populations of background microbiota in cheese brines treated with 100 ppm H_2O_2



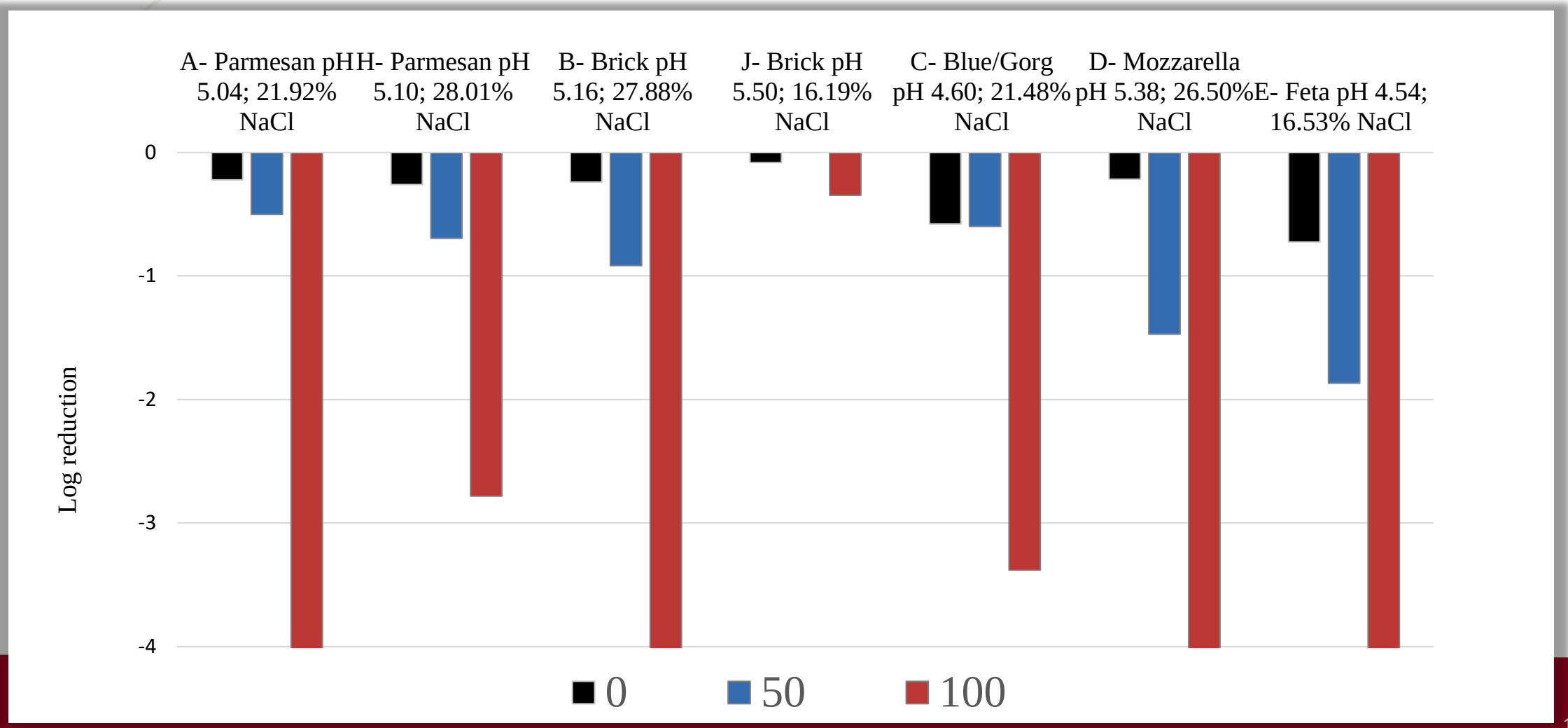
L. monocytogenes inactivation in 7 brines over 1 week with differing levels of H_2O_2 addition at **12.8˚C (55˚F)**



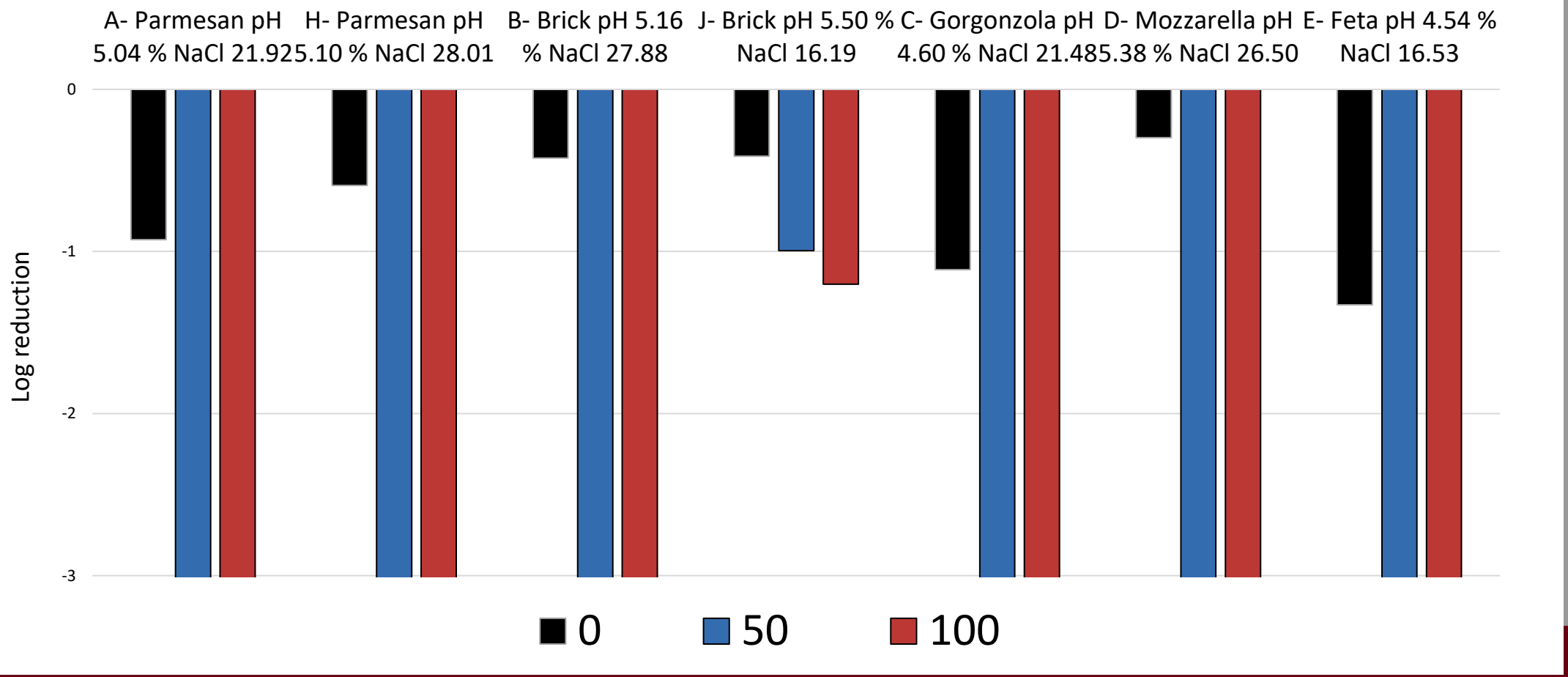
L. monocytogenes inactivation in 7 brines over 1 week with differing levels of H_2O_2 addition at **7.2˚C (45˚F)**



L. monocytogenes inactivation in 7 brines over 1 week with differing levels of H_2O_2 addition at **0°C (32°F)**



Staphylococcus aureus inactivation in 7 brines over 1 week with differing levels of H_2O_2 addition at **12.8°C (55°F)**



Summary Phase 2 – Commercial Brines

- No Seasonality observed with brine from different trials
- All Brines EXCEPT BRICK J
  - At 12.8˚C and 7.2˚C (*L. mono only*), a >4-log (*L. mono*) or 3-4 log (*S. aureus*) reduction was observed after 1 week when 100 ppm of H_2O_2 was added
  - At 0˚C, the reduction after 1 week varied between brines when 100 ppm of H_2O_2 was added
- Only 1 day after 100 ppm H_2O_2 was added, pathogen reduction varied between brine types and was not significant
- When only 50 ppm of H_2O_2 was added, there was variation in reduction between brine types after both 1 day and 1 week (all temperatures)

Brine J- Brick (pH 5.40-5.55, 10-19% salt)

- This brine did not show a reduction of *L. monocytogenes* with the addition of 100 ppm H_2O_2.
- This phenomenon was not seen with the other Brick brine tested
- Large populations of yeast (and sometimes lactic acid bacteria and other aerobic bacteria) were detected in uninoculated samples of this brine

- Our theory is that catalase+ yeasts can inactivate H_2O_2 and prevent *L. monocytogenes* inactivation

Overall Conclusions

- Fresh/Commercial brines without hydrogen peroxide pathogens survived well for 7 days (<1 log decrease) (pH 4.6, 20% salt, 15.6˚C)
- At a given pH, fresh brines with higher salt concentration (20%) accelerated inactivation when 50 or 100 ppm H_2O_2 was added.
- Pathogen inactivation in stressful conditions is likewise enhanced by higher storage temperature.

Log reduction of Listeria monocytogenes in brine with 50 ppm H_2O_2	
12.8˚C	3-4
7.2˚C	1.4-4.3
0.0˚C	0.5 -1.9

Overall Conclusions

- Other brine components (whey, fat, protein, microbiota) can potentially impact inactivation
  - Fresh, filtered brine: >4.8 log reduction of *L. monocytogenes* with 50 ppm v.s. 100 ppm to achieve similar reduction in commercial brines
- Type of background microbes present in brine is important
  - High catalase activity can reduce efficacy of hydrogen peroxide in brine
- Monitoring hydrogen peroxide levels after addition and at sufficient intervals to ensure target activity is maintained is critical

Additional Considerations

- Evaluate the effect of hydrogen peroxide on the organoleptic properties of cheese during brining
- Hydrogen peroxide is an oxidizing agent
  - May cause corrosion of low-grade stainless steel especially at high concentrations.
  - Suggested use of high grade 316 stainless steel, fiberglass or sealed concrete
  - Low dosage of 100 ppm as used in this study would minimize the potential for vessel corrosion.

Acknowledgements

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  - Tim Stubbs, Chad Galer
  - Dennis Seman
  - Wendy Bedale

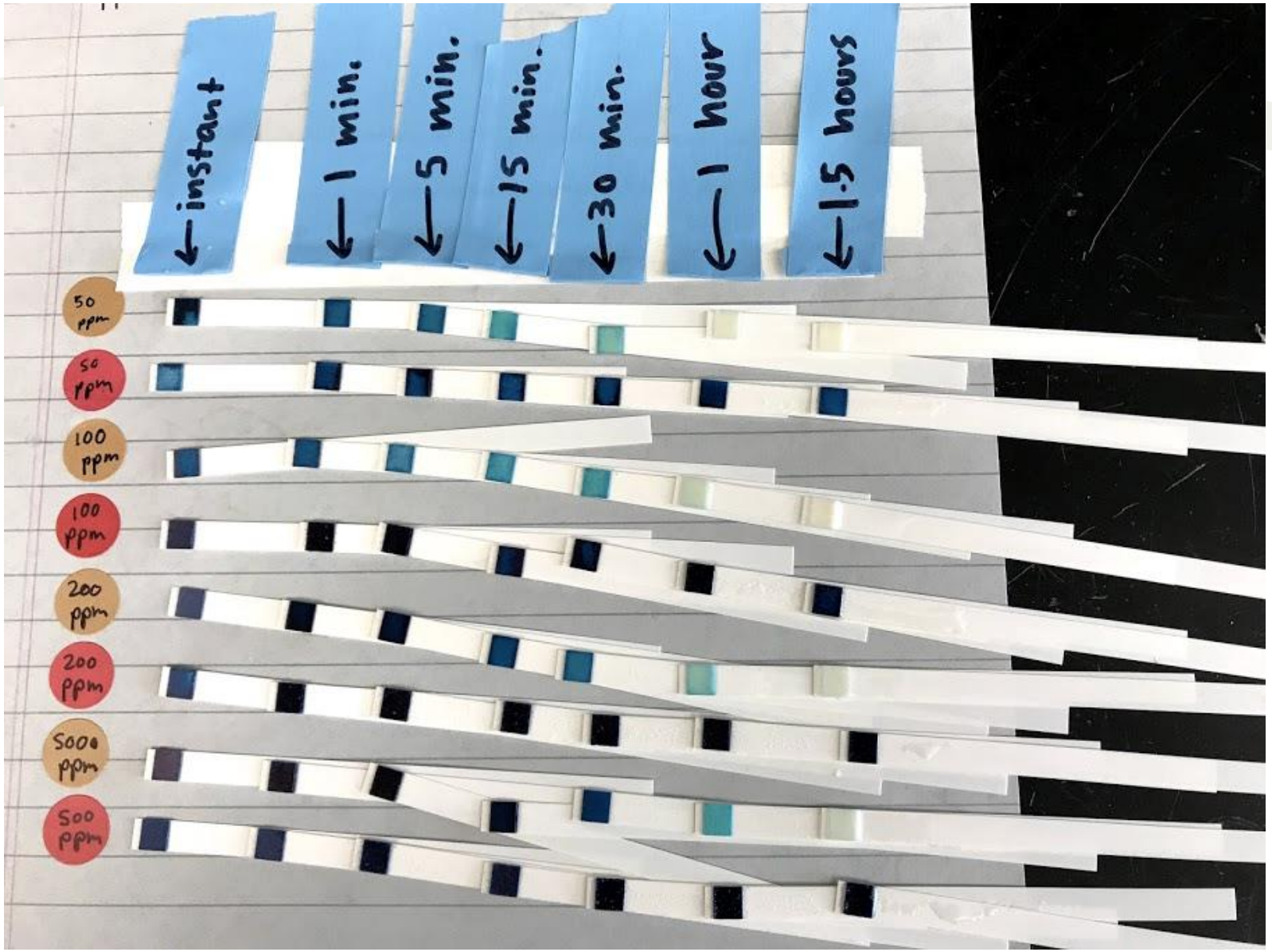
Take-home messages

- No “seasonality” observed with brine from different trials
- Each brine is unique
  - The two gorgonzola and two brick brines did not behave in the same way
- pH and salt level were not the only driving factors influencing *L. monocytogenes’* ability to survive/thrive
- 100 ppm of H_2O_2 can be an effective way to reduce *L. monocytogenes* in one week as long as tests are performed to ensure the 100 ppm level of H_2O_2 persists

H_2O_2 Testing

A comparison between how long H2O2 stays active in each of the 2 brick brines

Red= Brine B
Brown= Brine J



Methods

- Each brine inoculated with 5-6 log *L. monocytogenes or* 4-6 log *S. aureus* and dispensed into 14 ml tubes
- Samples were incubated at the appropriate temperature (12.8C, 7.2C, or 0C)
- Three inoculated samples were tested for each brine for each temperature (2 samples for 0C) at time 0, 1 day, and 1 week
- Two uninoculated samples were tested for each brine for each temperature at the time 0, 1 day, and 1 week
- pH was also measured at each sampling point for both inoculated and uninoculated samples

Methods continued

- Inoculated samples were plated on Modified Oxford agar with Trypticase Soy agar overlay (T-MOX, 35C for 2 days) or Baird Parker agar with Trypticase Soy agar overlay (T-BP, 35C for 2 days)
- Uninoculated samples were plated on:
  - Total plate count- Lactic acid bacteria-
  - Yeast/mold-