Emerging Topics: Inhibition and Lethality Technologies to Control Bacterial Pathogens in Dairy Products

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Overview

- What are the pathogens risks and how can they be managed?
- *Listeria* control in soft cheeses and brine
  - pH and acid type
  - Protective cultures
  - Fermentate
  - Hydrogen peroxide
- *Staphylococcus* control in brine and whey by hydrogen peroxide
- Thermal inactivation for *Listeria* and *E. coli* in cheese milk
- Summary

What are the risks? Behavior of pathogens

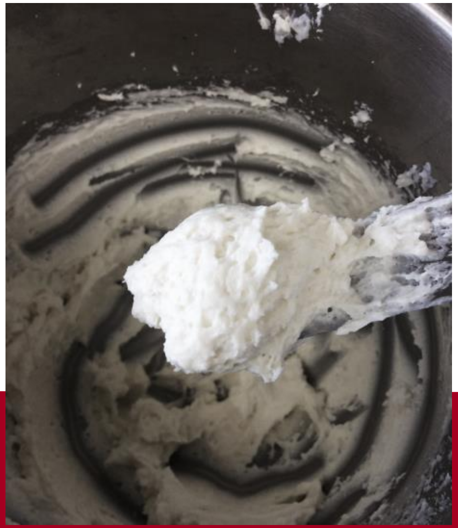
- In cheese during ripening and storage
  - Soft/semi-soft cheese: Pathogens may grow
  - Hard/semi-hard cheese: Pathogens are slowly inactivated
    - *Listeria* (up to 20 months in Cheddar held at 45°F)
    - *E. coli* O157:H7 (160 days in Cheddar; 180 days in Gouda)
- In brine
  - *Listeria* salt tolerant; can survive for months in brine
  - Brine may be reused for years
- In whey
  - Cooling may take hours
    - If neutral pH, potential for growth; if pH drops, poor quality whey

Background: Mitigating risks in high-moisture cheese

- Driving factors for safety
  - Water activity
    - Function of moisture, salt, protein, etc.
  - pH/acidity
    - Differences among acid types
  - Competitive microflora
    - Starter bacteria
    - Non-starter lactic acid and spoilage bacteria

Testing mitigation strategies: Developing a model cheese system

- Represent direct acidified soft cheeses (Hispanic-style, ricotta, mozzarella) with no starter culture
- Micellar casein, cream, lactose, water, 1.25% salt, acid
- Inoculate with *Listeria monocytogenes,* vacuum package
- Store at 4°C for 8 weeks; test weekly



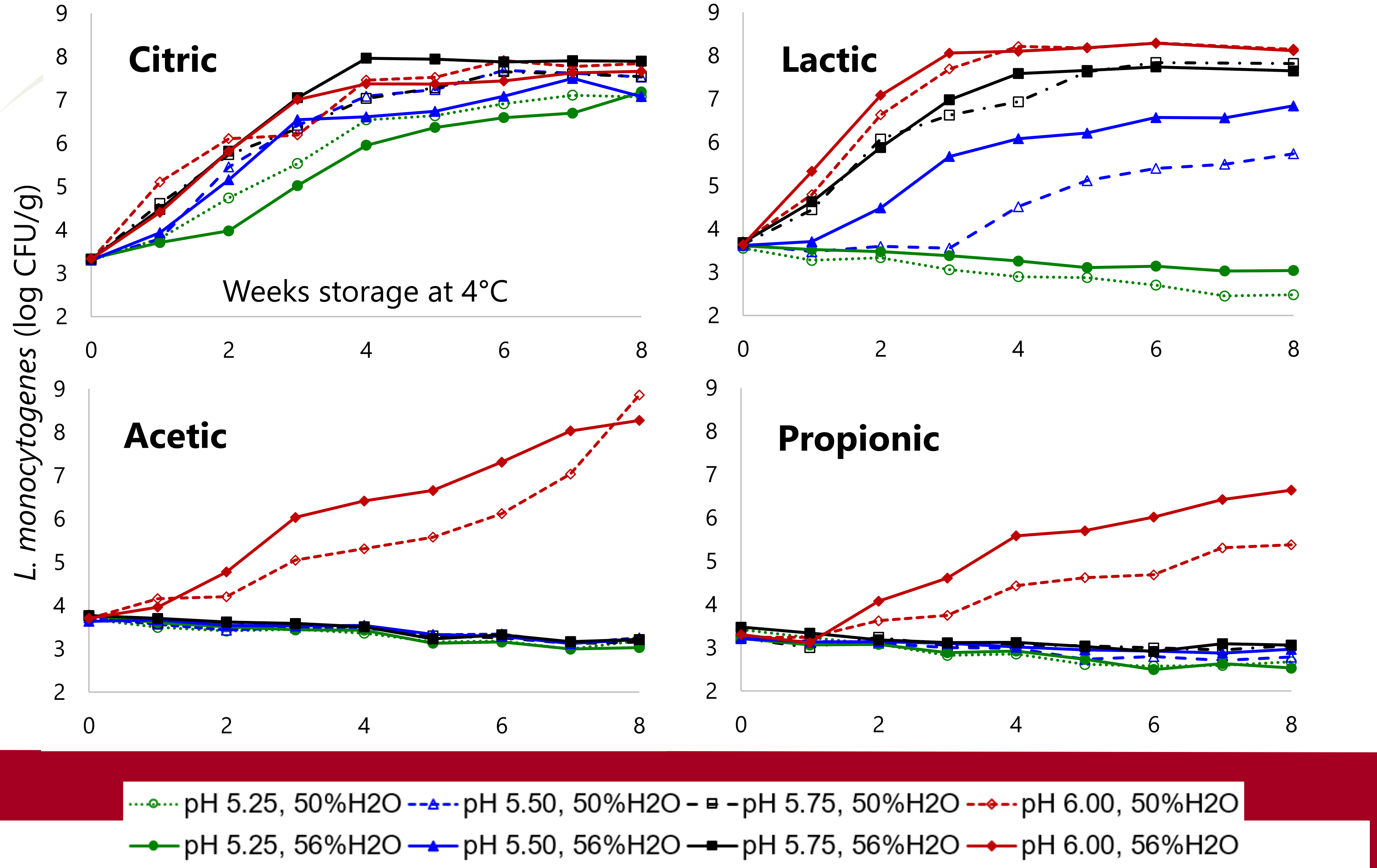
Identify formulations to inhibit *Listeria* in model soft cheese

- Phase 1: combinations of acid type, pH, and moisture
- Phase 2: fermentate (commercially available)
  - Label: cultured milk solids or cultured sugar-vinegar
  - Source of organic acids; potentially bacteriocins
- Phase 3: adjunct protective cultures (commercially available)

Phase 1: Moisture, pH, acid type

- 32 treatments x duplicate trials
  - 2 moisture values (50, 56%)
  - 4 pH values (5.25, 5.50, 5.75, 6.00)
  - 4 acid types (lactic, acetic, citric, propionic)

Acid type and pH on *L. monocytogenes* in cheese (pH 5.25-6.00, 50-56% moisture, 1.25% salt)

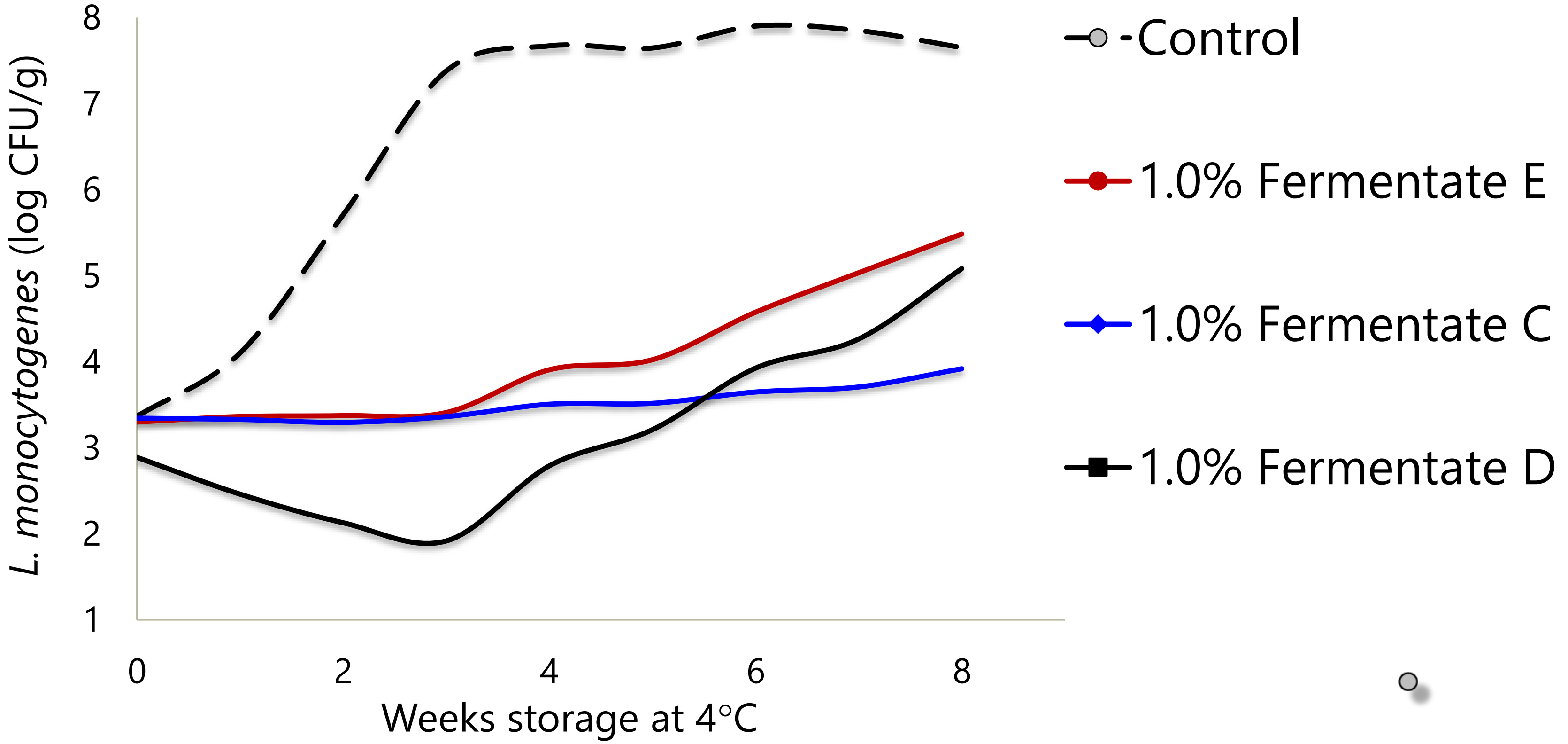


Phase 2: Inhibition with fermentates

- Labeled as cultured milk, cultured sugar-vinegar
  - Commercially available proprietary ingredients
- Fermentation byproducts depend on
  - Culture(s) used: *Propionibacterium, Lactococcus, Pediococcus,* etc.
  - Substrate
  - Controlled fermentation: Temperature, oxygen, nutrient availability
- Likely active compounds
  - Organic acids; single or blends (lactic, propionic)
  - Frequently blended with vinegar (acetic)
  - May or may not contain bacteriocin activity
- Challenges: Activity may vary between suppliers, within portfolio, between lots

Effect of cultured milk solids or cultured sugar-vinegar

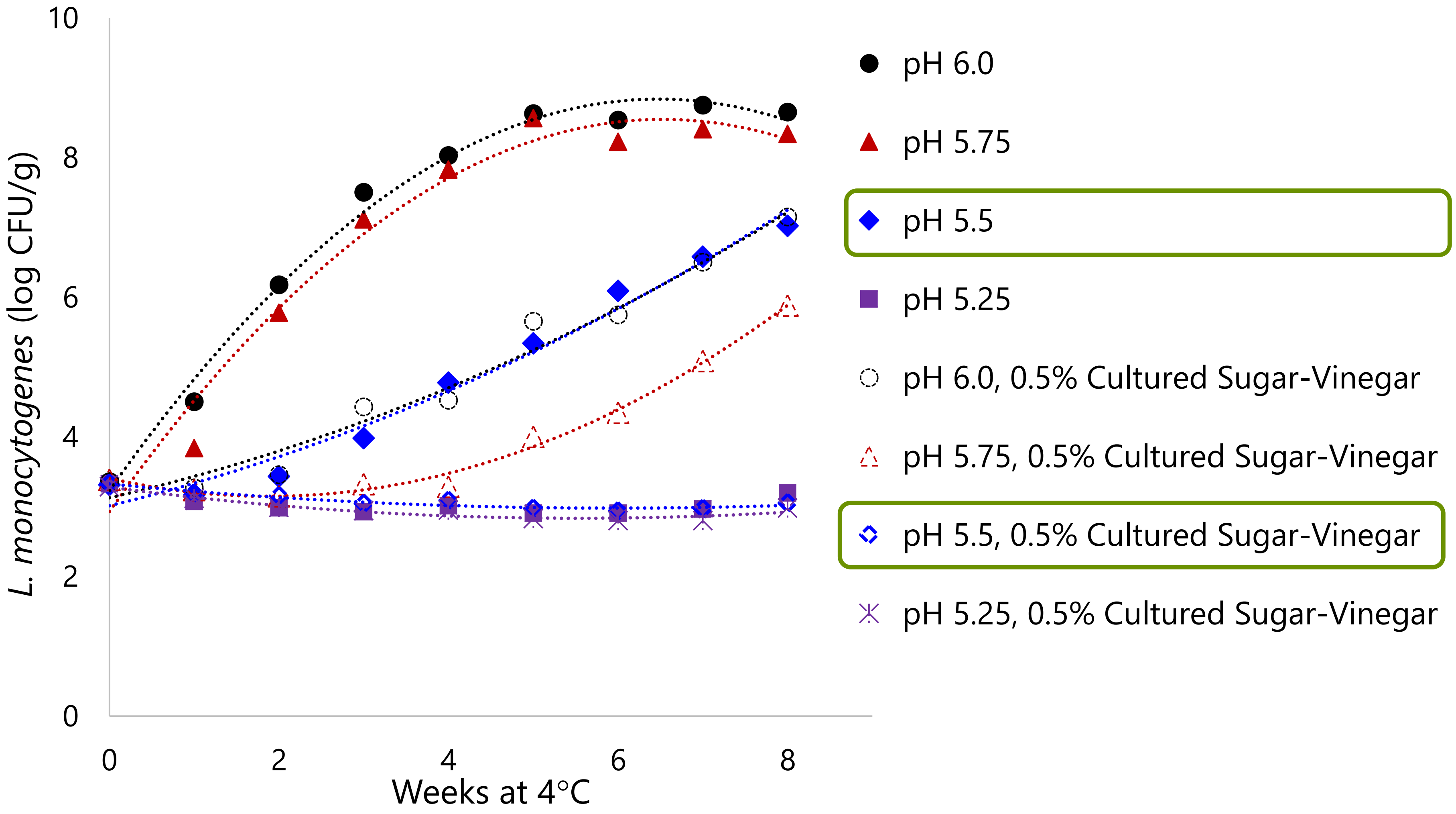
L. monocytogenes, pH 6.0, lactic acid, 56% moisture cheese



Fermentates labeled "cultured milk" (E and D) or "cultured sugar, vinegar" (C)
Engstrom, et al., manuscript in review

Fine-tuning formulations

L. monocytogenes in 56% moisture cheese acidified with lactic acid
Supplemented with 0.5% cultured sugar-vinegar Fermentate C



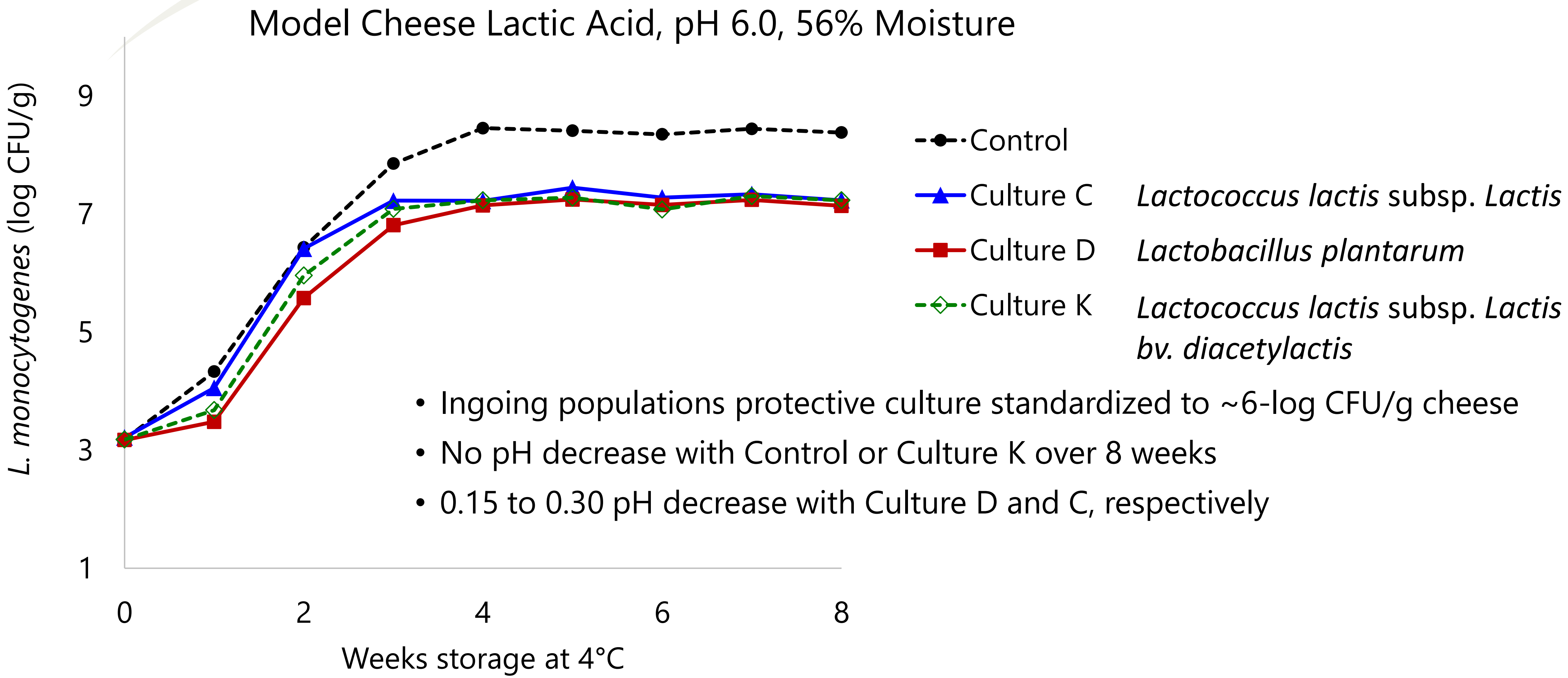
Anderson, et al., manuscript in review

Phase 3: Effect of protective cultures

- Added as starter culture or adjunct protective cultures
  - E.g.: *Lactococcus lactis, Lactobacillus plantarum*

- Produce acids, bacteriocins
- Compete for nutrients
- Can be used effectively in conjunction with other preservatives
- Effective when product may be temperature abused

Protective cultures don't always work if storage temperature (4°C/40°F) doesn't allow sufficient metabolism



- Ingoing populations protective culture standardized to ~6-log CFU/g cheese
- No pH decrease with Control or Culture K over 8 weeks
- 0.15 to 0.30 pH decrease with Culture D and C, respectively

Culture were previously shown to be active at 10°C; current study shows little activity at 4°C

Lessons learned

- Understanding effect of acid type and pH can direct control of *Listeria* in high moisture cheese
  - Propionic > acetic > lactic > citric
- Fermentates (cultured milk/cultured sugar) can delay growth, but effect greater in presence of vinegar (acetic acid)
  - Low levels of fermentate can improve efficacy of acids
- Protective cultures can inhibit listerial growth but must be actively metabolizing to be effective

Background: Pathogen risks in cheese brine

- Brine/salting important step in manufacturing cheese
- Brine can serve as a reservoir of salt tolerant pathogens
  - *Listeria monocytogenes* (survival up to 270 days in some brines)
  - *Staphylococcus aureus*
- Mitigation strategies to kill pathogens (clean break)
  - Ultrafiltration, pasteurization, ozone, superoxides
  - Sodium hypochlorite; hydrogen peroxide

% 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

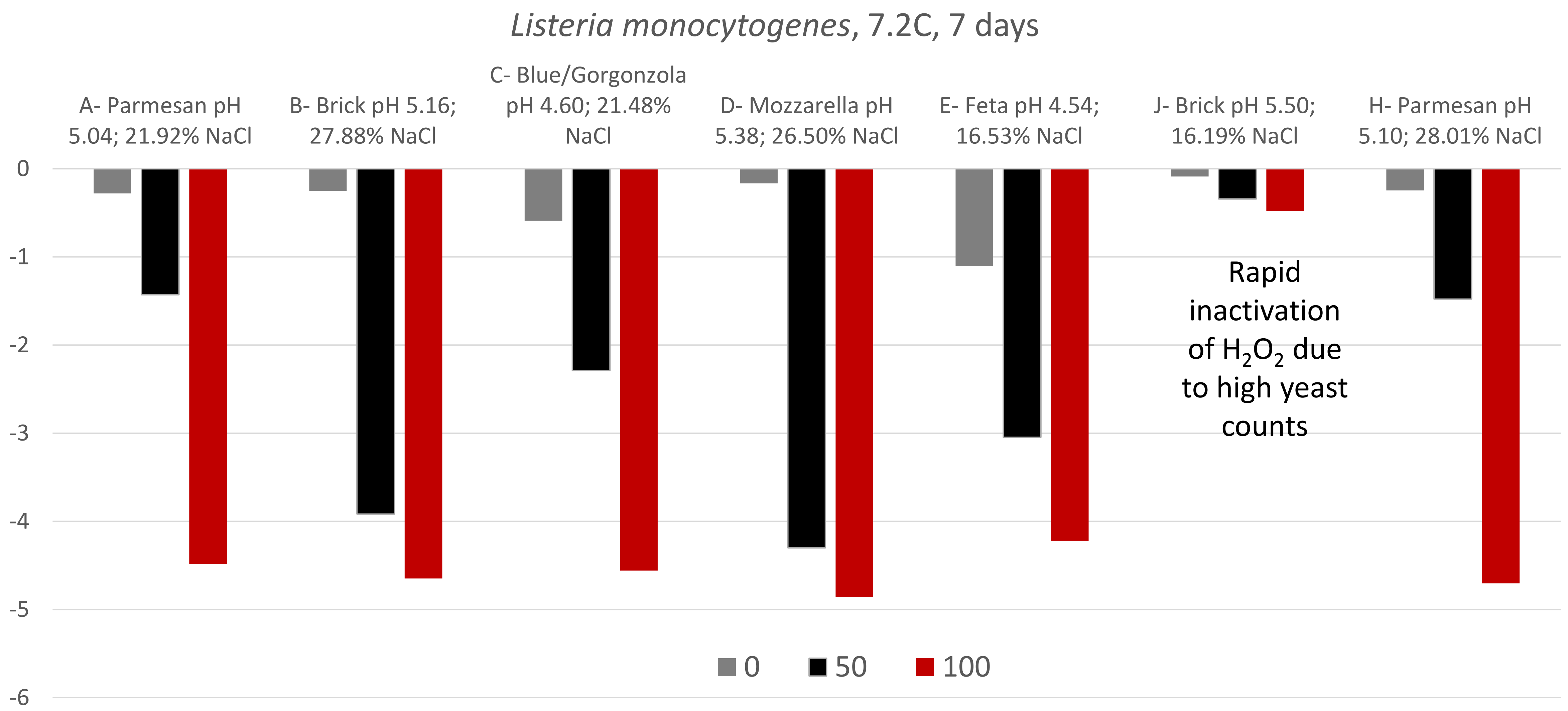
Effect of hydrogen peroxide in reducing microbial levels in cheese brine

- Preliminary work (*Listeria* only)
  - Fresh brine (lab prepared; sterile)
    - Variables: pH (4.6, 5.4), salt (10, 20%), temperature (50, 60°F), H_2O_2 (0, 50, 100 ppm)
  - Validated with 4 used unfiltered brines (pH, salt, cheese type)
    - Greater efficacy with higher salt; less activity if high mold counts
- Validation with used unfiltered brines
  - Variables: 7 brines (pH, salt, cheese type), temperature (32, 45, 55°F), H_2O_2
    - Three lots of each, seasonality
    - Naturally occurring bacteria, molds, yeasts
    - Added *S. aureus* at 55°F only

Commercial Brines Tested

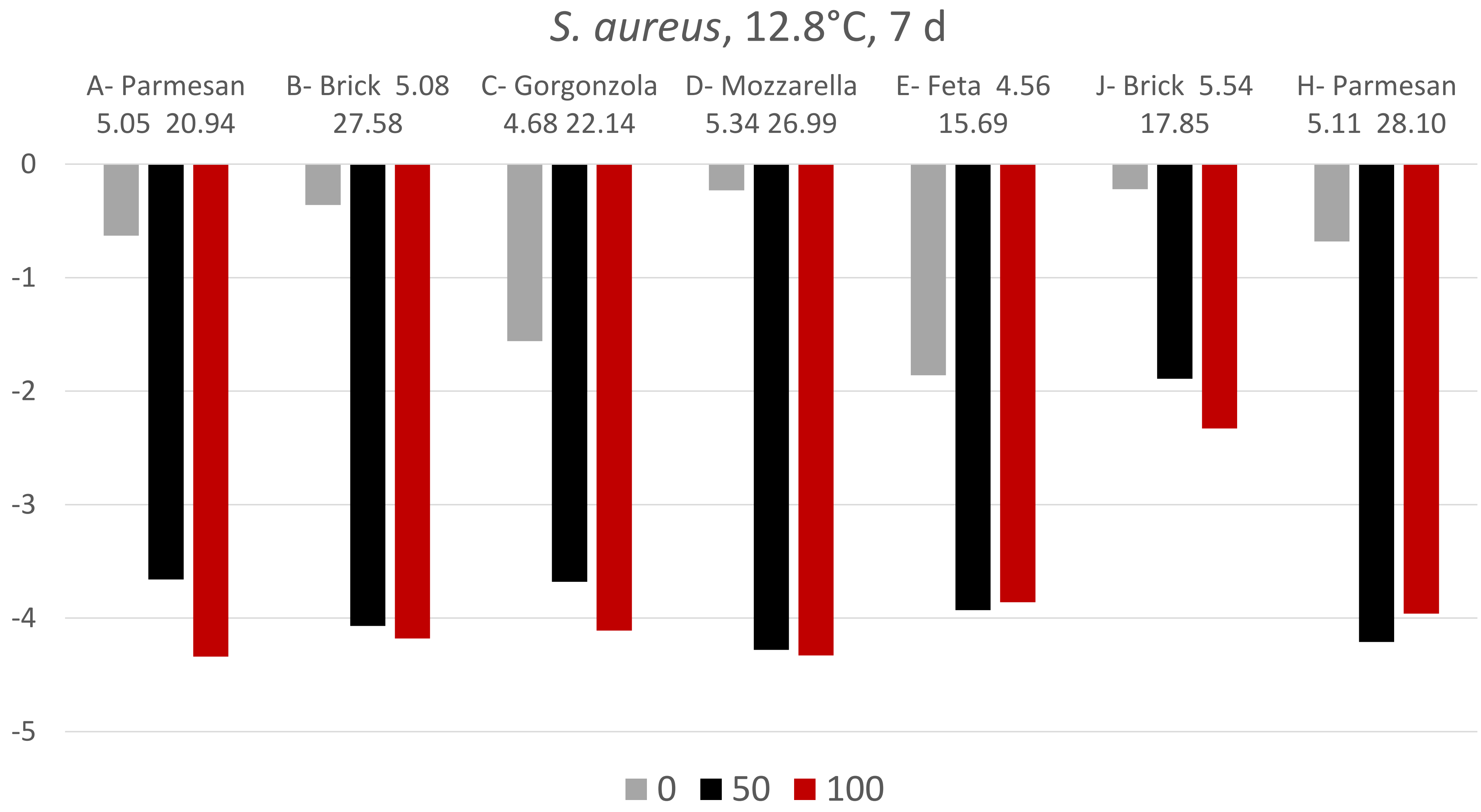
	Cheese Type	Use temperature	pH range for brine received	% Salt range for brine received	A_w range for brine received
B	Brick	71-73°F	5.08-5.20	27-29	0.766-0.777
J	Brick	40-45°F	5.43-5.54	12-19	0.867-0.909
C	Blue/ Gorgonzola	50-60°F	4.59-4.60	20-23	0.822-0.827
E	Feta	55°F	4.51-4.56	16-18	0.858-0.874
D	Mozzarella	30-32°F	5.34-5.40	25-27	0.788-0.797
A	Parmesan	50-53°F	5.03-5.05	21-22	0.819-0.838
H	Parmesan	53-55°F	5.10-5.11	27-29	0.761-0.768

Brines received August 2019, November 2019, January 2020
Aw $\geq$0.86 within growth range for *S. aureus* if optimal pH and temperature



Greater inactivation:

100 ppm H_2O_2; higher temperature; low levels of catalase + molds/yeasts



More sensitive than *Listeria* when under same conditions

Lessons learned

- Hydrogen peroxide effective in killing *Listeria* and *S. aureus* in brine
- Most consistent kill with 100 ppm H_2O_2
- Temperature affected inactivation
  – 100 ppm H_2O_2 @1 week
    - 12.8 and 7.2°C: >4-log reduction
    - 0°C: 2.5->4 log reduction, varied between brines
- Catalase + microbes can inactivate H_2O_2
- H_20_2 levels must be monitored for effective levels

Background: Whey Manufacture and Use

- Liquid byproduct during cheese manufacturing
  - Uses: Protein powder, whey cheese, baby formula, etc.
  - Whey pH is often 5.9-6.6
- Regulations
  - *"If holding > 4 hours, then must either be at <45°F or >140°F."*
  - Time starts at the draw from vat; cooling capacity limited
- Food safety issue
  - Growth of pathogens
    - Repasteurization will kill other vegetative microbes
  - Production of heat stable toxin by *Staphylococcus aureus*
    - Optimal growth pH 6.5; Temp 86-99°F (30-37°C); poor competitor
- Quality versus food safety issue
  - Low pH = *S. aureus* inhibition = Unacceptable product

Objective: Determine the inhibition of *S. aureus* by hydrogen peroxide

- Multiple lots whey obtained from commercial cheesemaking
  - 4 no starter, 2 mesophilic starter, and 2 thermophilic starter
  - Average pH 6.3-6.6
- 3-strain mixture of *Staphylococcus aureus* added to whey
  - Inoculated at 3-log CFU/ml
- Hydrogen peroxide added
  - Each whey type contained either 0ppm, 10ppm, and 100ppm hydrogen peroxide
- Incubation at 70°F or 90°F
- Enumerated in duplicate at 0, 4, 8, 12, and 24 hours on Baird-Parker agar (selective) overlaid with Tryptic Peptone agar (non-selective) to enhance recovery of injured cells

Summary of *S. aureus* Enumerations

NO STARTER WHEY	70°F	90°F
0ppm Hydrogen Peroxide	Growth >1 log	Growth >2 log
10ppm Hydrogen Peroxide	No Growth	Growth >2 log
100ppm Hydrogen Peroxide	Decrease 2 log	Decrease >2 log

STARTER WHEY	70°F	90°F
0ppm Hydrogen Peroxide	No Growth	Decrease 2 log
10ppm Hydrogen Peroxide	No Growth	Decrease 2 log
100ppm Hydrogen Peroxide	Decrease >1 log	Decrease 2 log

Lessons learned

<u>Whey with Starter Culture</u>

- Starter culture competitively inhibits the growth of *Staphylococcus aureus* at 70°F and 90°F for up to 24 hours
  - pH reduction may adversely affect quality attributes of whey
  - Without hydrogen peroxide, pH decrease from ~6.4 to 4.4

<u>Whey with No Starter Culture</u>

- Require time-temperature control and/or the addition of hydrogen peroxide
  - Safe up to 24 hours at 70°F
  - Use $\geq$10ppm hydrogen peroxide if stored at 90°F for < 8 hours
  - **<u>Use 100ppm hydrogen peroxide if stored at 90°F for > 8 hours</u>**

Manuscript in preparation

Background: Heat Treatments of Milk for Cheese

- Thermized milk for cheese
  - Below pasteurization temperatures
    - 145–149 °F (63–65 °C) for 15 seconds
  - Not technically raw, but is technically unpasteurized.
  - Reduces heat-sensitive pathogens
  - Other heat-resistant beneficial microbes may survive

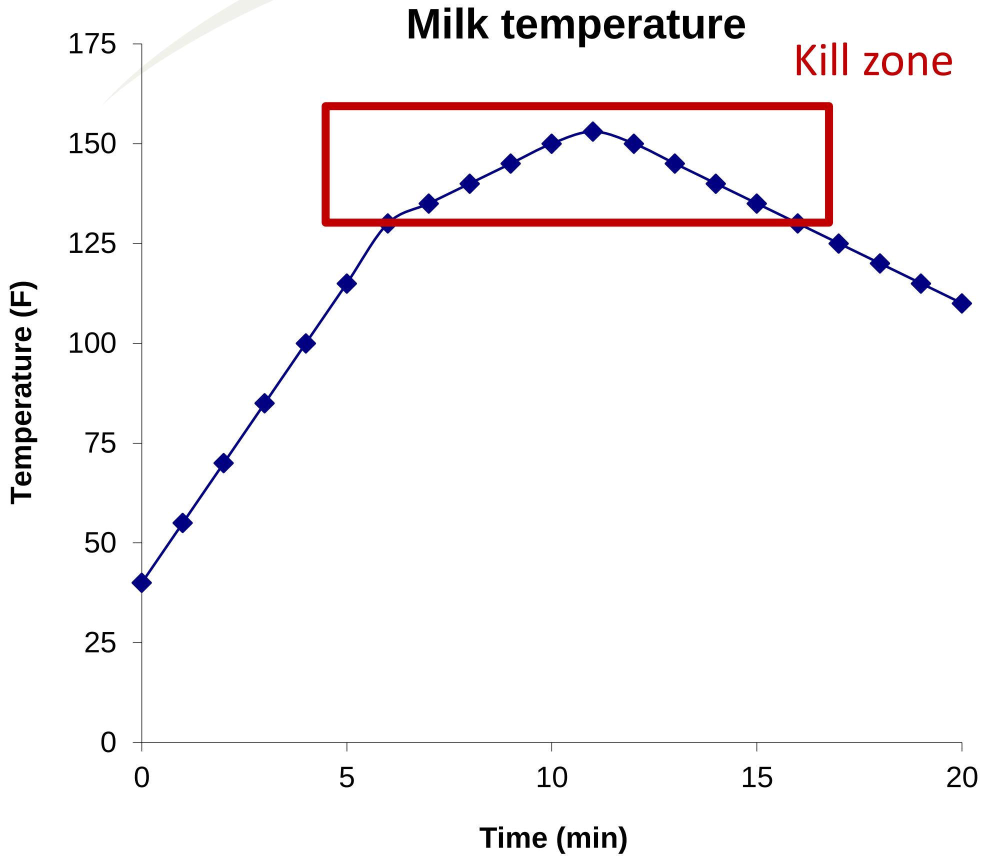
- Pathogen inactivation will continue during aging of hard cheese
  - High moisture cheese can support growth of *Listeria* if it were present
- Heating of milk does not necessarily have to be conducted in sealed/timed equipment to be effective, but must be controlled

How much kill is needed?

- Depends on
  - Type of pathogen (infectious dose)
  - Type of cheese
    - Moisture
    - pH
    - Acid type
    - Salt content
    - Starter culture activity
  - Time/temperature conditions for ripening/aging
- Requires other process controls for formulation and storage

	L. monocytogenes (min:sec)	*STEC* (min:sec)
D-value – 150°F (65.6°C)	0:17	0:07
Est time 3-log reduction – 150°F	0:51	0:21
D-value – 145°F (63.8°C)	0:33	0:17
Est time 3-log reduction – 145°F	1:39	0:51
D-value – 140°F (60°C)	2:27	1:00
Est time 3-log reduction – 140°F	7:21	3:00
Z-value	11.0°F / 6.1°C	11.0°F / 6.1°C

What can we do if we collect the right data?



Reference Temp	150	°F
D value =	1.0	min
z value =	10	°F
Start temp	40	°F
Maximum temp	153	°F
Hold time @ max	1	min
End temp	110	°F
Entire process	20	min
Log kill	**4.9 log**	

For illustration purposes only. DO NOT USE FOR VALIDATION

Lessons learned

- Heating required to kill STEC is less than what is required for *Listeria*
  - Therefore, heating for milk used for aged hard cheese may be less than what is needed for soft cheese
- Data collected is applicable to bovine milk, but may be different for sheep or goat milk, depending on fat content

Summary

- Multiple strategies to control bacterial pathogens in dairy products while still allowing flexibility for production, formulation, cooling
- Acid types, fermentates, competitive microbiota holds promise to inhibit Listeria in soft cheeses
- Hydrogen peroxide useful in brine, whey, and surface of cheese
  - H_20_2 has also been effective to control *Listeria* on surface of soft cheeses (D'Amico lab, U Conn)
- Sub-pasteurization thermal treatments can eliminate pathogens in cheeses made with unpasteurized milk
- Work with producers and regulators to encourage use of innovative technologies to ensure safety

Thank you for your attention.
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