Control of Sporeforming Bacteria in RTE Foods during Cooling and Storage

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Overview

- Spores of concern
- Factors to control outgrowth
- Validation of formulation and temperature control
  - Predictive models
  - Indicator and surrogates
  - Extended cooling of meats
  - Sous vide foods
  - Shelf stable, low-acid, formulation-safe foods
    - Process cheese
    - Coffee beverages

Spores of concern

- *Clostridium perfringens*
  - Toxicoinfection; ingestion of high number of cells (10^6 -10^7 cells; sporulation in intestine releases toxins
  - Associated with improper hot-holding or during extended cooling
- *Bacillus cereus:*
  - Emetic, diarrheal toxins (requires 10^6 -10^7 cells per gram)
  - Associated with improperly cooled and stored foods (rice, pasta, custards, soups, meats, vegetables, dairy)
- *Clostridium botulinum*
  - Potent neurotoxin (level of growth or total populations not known)
  - Associated with low acid foods stored at non-refrigeration temperatures
- Cooking kills vegetative cells but spores and toxins unaffected
  - Potential for spore outgrowth during cooling and storage

Conditions that select for pathogenic spores

- Sous vide cooking
  - "Under vacuum"
  - Typically, low temperature-long time
  - Reduces or eliminate vegetative microbes
  - Most spores survive
  - Some processes, particularly vegetables, include a "nonprot Cbot cook"
    - 90°C, 10 min
  - Process results in reduced oxygen in intact packages

- Reduced Oxygen Packaging
  - Cooking/hot-fill
    - Including *sous vide*
  - Modified Atmosphere
  - Plastic or foil overwrap in combination with respiring microbes vs. competitive microbes
    - Fish/seafood; packaged fresh mushrooms
  - Select for anaerobic microbes e.g., *Clostridia sp.*
    - *Bacillus cereus* is a facultative anaerobe so can grow in ROP

Factors affecting microbial growth

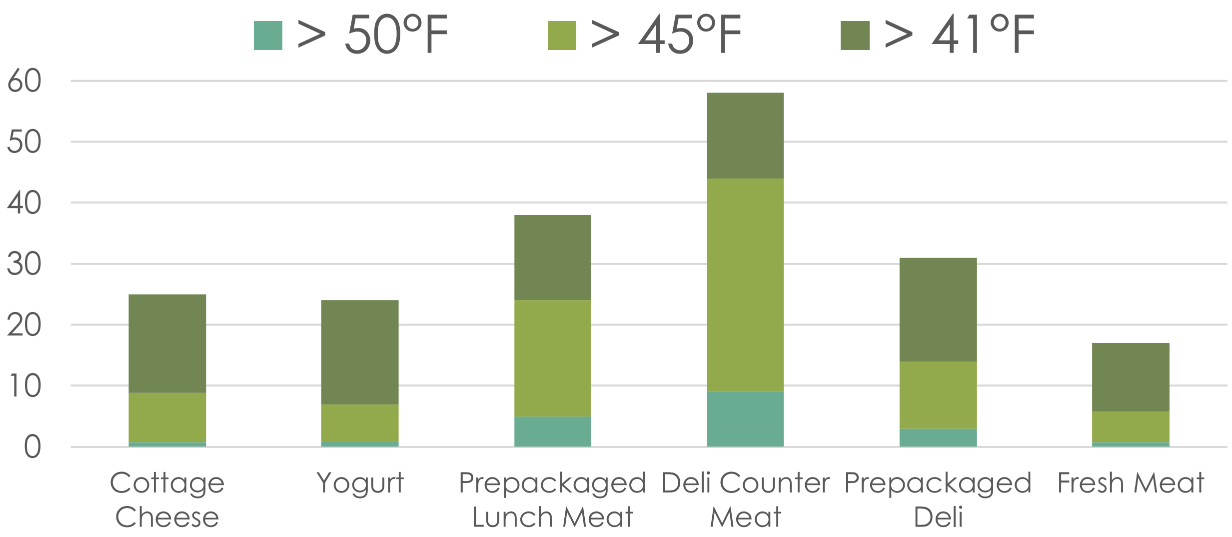
- Temperature, time
- pH (acidity)
- Water activity (function of moisture/salt)
- Other antimicrobials (growth inhibitors)

Effect of temperature on spore outgrowth

- Mesophilic *Clostridium botulinum* and *Bacillus cereus*
  - 10°C (50°F) minimum growth temperature under ideal conditions
    - But may take weeks to months grow and produce toxin
  - 12.8°C (55°F) accelerated growth
    - May take only 1 week to grow/produce toxin
- Psychrotrophic strains
  - Certain strains of *Bacillus cereus;* enterotoxin generally at > 10°C (50°F)
  - Non-proteolytic *Clostridium botulinum*
    - <u>VERY</u> slow at 3°C (37°F) under ideal conditions of pH/water activity
    - Faster growth at 7.2 and 12.8°C (45 and 55°F)
- *Clostridium perfringens*
  - <u>VERY</u> slow growth below 20°C (68°F)
  - Typically, a concern during extended cooling or improper hot-holding

Refrigeration alone not dependable

Retail



% of Products with Temperatures >41°F (4°C)

Home Refrigerator

Food Sample Temperatures (after 24h)	
Exceeding temperature	%
>41°F	17
>45°F	5
> 50°F	0.7
>55°F	0.2
>60°F	0.2

Ecosure 2007

Challenge studies should consider temperatures > 45°F

FDA Food Code/NACMCF Tables

Table A. Inhibition of sporeforming pathogens at non-refrigeration conditions

	Critical pH		
Critical A_W	**4.6 or less**	**4.6 to 5.6**	**>5.6**
0.92 or less	**Non-TCS**	**Non-TCS**	**Non-TCS**
>0.92-0.95	**Non-TCS**	**Non-TCS**	**Product Assessment Required**
>0.95*	**Non-TCS**	**Product Assessment Required**	**Product Assessment Required**

Non-TCS = does not require temperature control for safety

* *Clostridium perfringens* lower aw growth limit 0.97

Food Antimicrobials ("Preservatives")

Conventional	Clean Label
NaCl	Salt (Reduces available water, a_w)
Lactate, propionate	Cultured sugar, cultured milk
Diacetate, acetic acid	Vinegar (dry vinegar, buffered vinegar)
Nitrite	Cultured celery (convert nitrate to nitrite)
Sorbic acid	Rowanberries
Nisin (bacteriocins)	Culture sugar/ dairy solids
Phenolics, flavonoids	Fruit / spice extracts; coffee extracts
Lactic acid bacteria starter cultures	Protective cultures Competitive microflora In situ acid/bacteriocin production

No single formulation strategy works for all foods

Validation of inhibition of sporeforming pathogens

Getting Started: Use Predictive models

- Pathogen Modeling Program; ComBase*
  - ComBase Perfringens Predictor typically accurate for foods without antimicrobials
    - Validated only in meats, but useful in evaluating cooling deviations
  - *B. cereus* model only limited to temperatures that are below optimal growth temperatures
  - C. *botulinum* based on growth and not toxin production
    - NEW ARS Prot Cbot model for cooked uncured ground beef
    - NP Cbot enumeration techniques inaccurate

Both are currently managed by ARS

Predictive models

- Validated process cheese-C. *botulinum* model by Tanaka et al., 1986
  - Accurate for standard of identity process cheese sauces
  - 2017 model by Glass et al., for non-standard cheese sauce is being validated and not in Excel form
- Other published C. *botulinum* models not "user friendly"
  - Meng and Genigeorgis (1993), NP Cbot, lactate, salt, temperature
  - Koukou et al., (2021), NP Cbot, 8 factors

Surrogates or indicators organisms

- *Clostridium sporogenes* PA3679: thermal surrogate for mesophilic (proteolytic) *Clostridium botulinum* (canning)
  - Genetically, a mesophilic non-toxigenic C. *botulinum*
    - Not a validated surrogate for growth inhibition
    - Need to be careful of the "real" PA3679 is used; some strains that are labeled PA3679 are not thermal resistant
  - Validation ongoing to compare growth inhibition with toxicity
- Other indicators: *Bacillus cereus* for screening
  - Grows faster than mesophilic C. *botulinum* at 12.8°C *(55*°F)
  - More sensitive to some antimicrobials such as nisin
- No appropriate surrogate for NP (psychrotrophic) C. *botulinum*

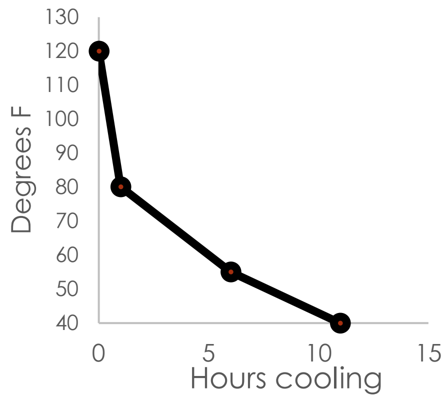
Growth comparison of pathogenic spores

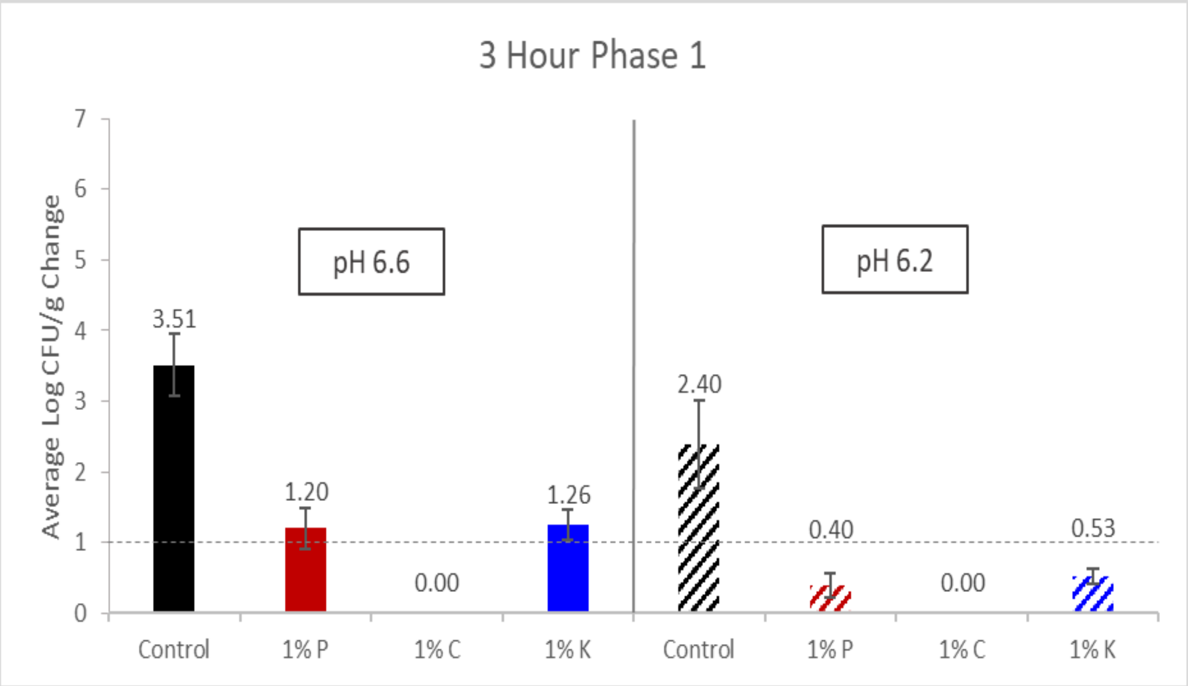
Uncured turkey, no antimicrobials, extended cooling

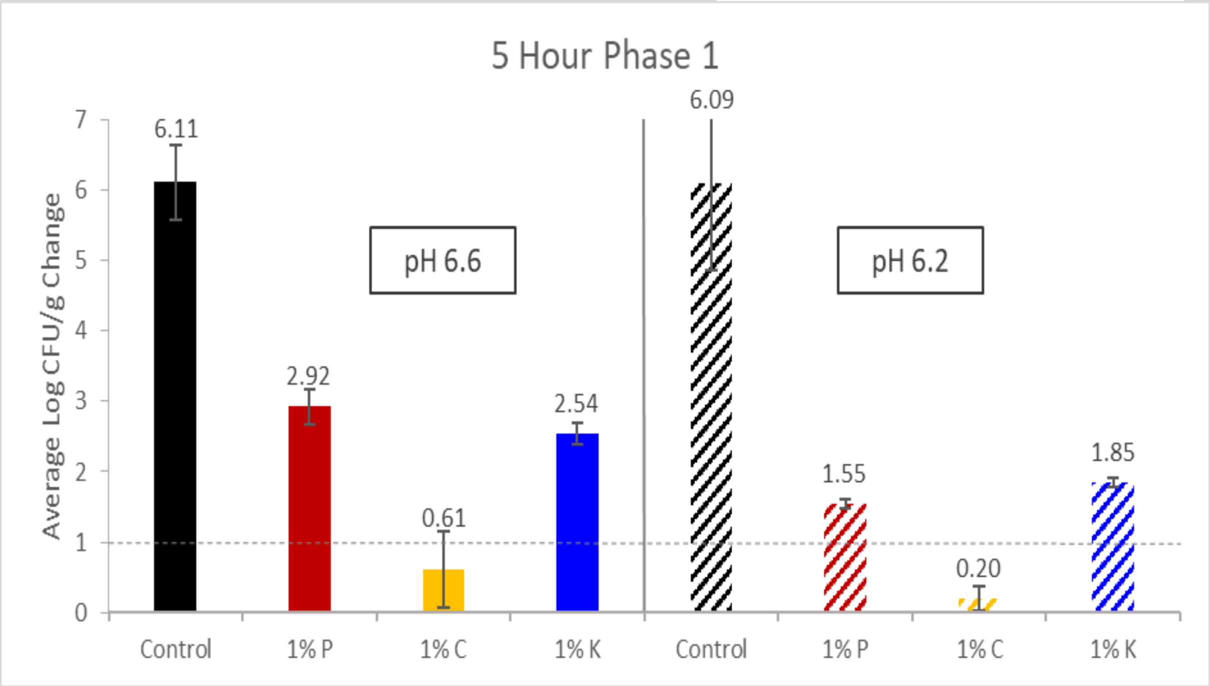
3-hour Phase 1 cooling (48.9-26.7°C/120-80°F); 5-hour Phase 2 cooling; 5-hour Phase 3 cooling; 75% moisture product

	pH 6.6 1.2% NaCl	pH 6.6 1.5% NaCl	pH 6.6 1.8% NaCl
	Average log increase over 0-time		
C. perfringens	3.34	3.16	2.66
B. cereus	1.12	0.93	0.87
C. botulinum	-0.09	0.01	-0.10
Maximum log increase 0.88 log, no toxicity detected in 270 samples assayed			

Effect of antimicrobials on C. *perfringens*
Uncured Turkey: 75% moisture, 1.5% NaCl
Extended Phase 1 cool (120-80°F),1, 2, 3, 4, 5 h







P: 1% Dry vinegar
C: 1% Dry vinegar-cultured sugar blend
K: 1% Dry vinegar – fruit-spice blend

Effect of temperature on microbial growth and toxin production in *sous vide* eggs

Sous vide eggs (no antimicrobial, pH 5.8, 0.5% NaCl, aw 0.99)			
	Clostridium botulinum (NP/P)	Bacillus cereus	Spoilage bacteria
7.2°C	No toxicity 16 wk	No growth 16 wk	0-6 log increase at 4 wk
			≤0.3 pH unit decrease
12.8°C	Toxic at 3 wk, but not 2 wk	3.2 log increase at 2 wk	0-4 log increase at 2 wk
			≤0.2 pH unit decrease

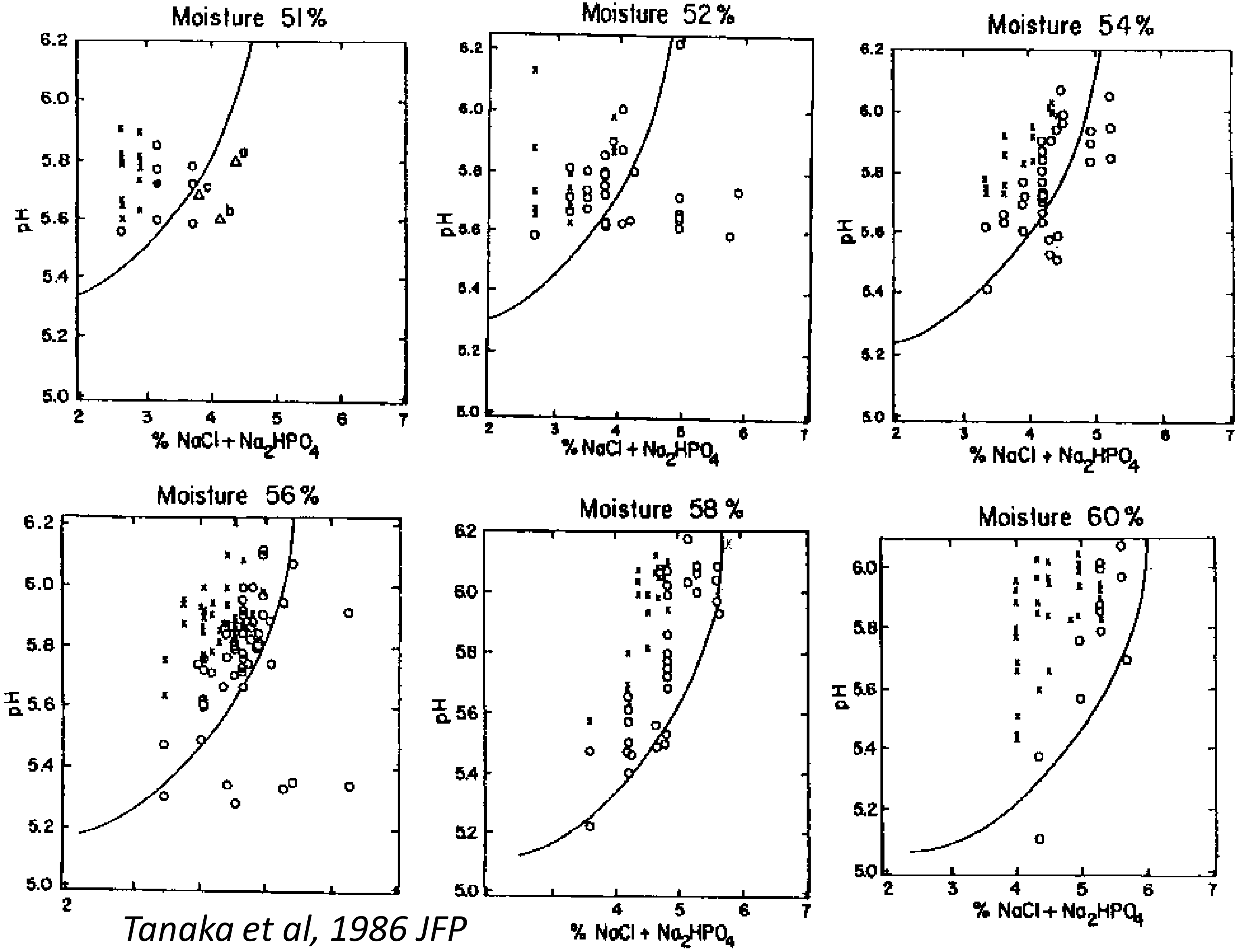
Take home lesson: Don't depend on spoilage microbes for inhibition

Effect of Dry vinegar, Fruit-Spice Blend, pH, temperature Time to toxin production NP-Prot C. *botulinum* *Sous vide* Uncured Chicken

		pH 6.15		pH 6.40	
Temperature °F (°C)	Testing Frequency	Control	0.5% Dry Vinegar + 0.6% Fruit-Spice-Vinegar blend	Control	0.5% Dry Vinegar + 0.6% Fruit-Spice-Vinegar blend
77 (25)	Daily	2 d	4 d	2 d	3 d
55 (12.8)	Monthly	1 mo	1 mo	1 mo	1 mo
45 (7.2)	Monthly	>6 mo	>6 mo	1 mo	3 mo

Antimicrobial delivered via injection of whole muscle
Cooked at 170F, 30 minutes
Glass et al., unpublished data

1986 FRI Model (*aka* Tanaka Model)



Tanaka et al, 1986 JFP

Validated predictive model for safety of shelf-stable process cheese spreads (>50% cheese, ~20% fat)

Moisture, pH, salt, phosphate emulsifier

2017 model by Glass et al. for shelf-stable process cheese sauces with >15% cheese

% moisture	pH	% NaCl	% DSP anh.	total salts	fat	% sorbic acid	Predicted Time (Weeks) to Failure
50.0	5.8	2.4	1.6	4.00	20	0	8
50.0	5.8	2.4	1.6	4.00	20	0.1	88
50.0	5.8	2.4	1.6	4.00	20	0.2	917
55.0	5.8	2.4	1.6	4.00	20	0	5
55.0	5.8	2.4	1.6	4.00	20	0.1	29
55.0	5.8	2.4	1.6	4.00	20	0.2	188

TABLE 4. *Model parameter significant effects (P < 0.10)*[a]

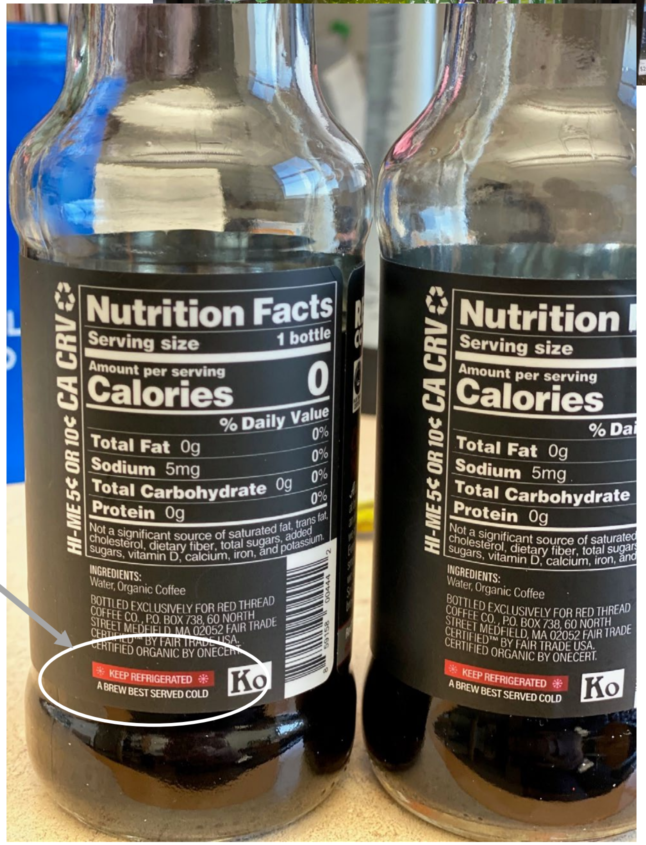
Variable[b]	χ^2	Prob > χ^2
pH	101.52	<0.0001
Sorbic acid	85.65	<0.0001
Moisture	46.59	<0.0001
DSP × DSP	31.62	<0.0001
NaCl eq	31.18	<0.0001
DSP	27.31	<0.0001
pH × sorbic acid	19.86	<0.0001
Moisture × sorbic acid	17.78	<0.0001
NaCl eq × sorbic acid	8.25	0.0041
Moisture × fat	7.67	0.0056
Fat	5.72	0.0168
Moisture × NaCl eq	4.26	0.0390
Moisture × pH	3.79	0.0516
K replacement	3.76	0.0526

[a] All linear and quadratic (curvilinear) and pairwise effects were considered for model fit.
[b] All variables are percentages, except pH.

Excel model will be available in Summer 2022

Coffee beverages

- Surge of canned/bottled coffee beverages
  - Hot and cold brew; nitro coffee; flavored; enhanced with nutritive and non-nutritive sweeteners, milk or milk substitutes
- pH >4.6; a_w >0.95; no added antimicrobials
  - Nitro coffee or hot-fill reduce oxygen content
  - No clear evidence of microbial spoilage
- No evidence that beverages are free of microbes, particularly cold brew
- Typically refrigerated, but not clearly marked
  - Cans/bottles mimic shelf-stable beverages
  - Some brands are validated to be shelf-stable
- Concerns with long term temperature abuse and hence with *Clostridium botulinum*



Antimicrobial properties of coffee beverages

- Factors affecting stability
  - Roast
  - Extraction temperature and time
  - Concentration (brix)
  - pH
- No predictive model for safety
- Validation studies specific for given product
  - Ex. Extraction at ~70F, 11 hours, final 2.2 Brix, pH 5.4 stable through 9 months, but 1.5 Brix toxic at 4 months

Summary

- Refrigeration alone will not guarantee safety
  - Formulation part of the well-designed food safety system
- Factors to consider
  - Temperature during production, distribution, at retail, consumers
  - Water activity, pH/total acidity
  - Synthetic and clean label antimicrobials
- Validation needed to confirm preventive controls work as intended
- Success also depends on clear labeling and education and cooperation of consumer for safe food handling

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Thank you for your attention

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