Recommendations for Designing and Conducting Cold-Fill-Hold Challenge Studies for Acidified Foods

Sponsored by the ***University of Wisconsin*** *and*
IAFP's Beverages and Acid/Acidified Foods Professional Development Group

Moderator: Elizabeth L. Andress - University of Georgia, Athens GA

Speakers:

Fred Breidt - USDA/ARS, Raleigh NC

Barbara Ingham - University of Wisconsin, Madison WI

International Association for Food Protection® WEBINAR

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Goals

- Review U.S. regulations covering acidified foods
- Describe how challenge studies can support safe processing of acidified foods
- Describe research-based recommendations for designing and conducting challenge studies for cold-fill-hold acidified foods
- Review elements that should be included in a challenge study report or scheduled process

Federal regulations

- Definitions (21 CFR part 114)
  - Acid foods have a natural pH of 4.6 or below
  - Acidified foods are low-acid foods to which acids or acid foods are added to bring pH to 4.6 or lower and with a_w >0.85
- Some foods are exempt, e.g.
  - Refrigerated foods
  - Acid foods
  - Alcoholic or carbonated beverages

- Manufacture of acidified foods must ensure:
  - Vegetative pathogens of public health significance are destroyed
  - Spoilage organisms are destroyed or formulation prevents growth
- FDA approved school and process filing required

Ref: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=114

Acidified Food Safety

- Acidification of foods to pH 4.6 or below is critical to prevent the outgrowth of spores of *C. botulinum*
- Proper acidification plus mild heat usually ensure shelf stability

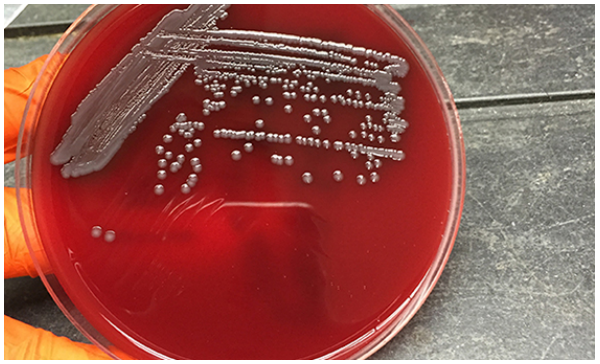
➔ Even mild heat may negatively affect some products

Limited existing research to support safe manufacture of cold-fill-hold acidified foods:

- Minimum cold-fill-hold in pickle brine pH $\leq$ 3.3
- Safety of products pH $\leq$ 3.8 formulated with specific levels of acetic and benzoic acid

➔Product-specific challenge studies can be used to establish safety of products/formulations

The Challenge Study



- Designed and evaluated by an expert microbiologist
- Specific for: given food product formulation and manufacturing practice
- Account for packaging and storage conditions.
- Adhere to Good Manufacturing Practices (GMPs).

➔Guidelines from the *National Advisory Committee on Microbiological Criteria for Food*

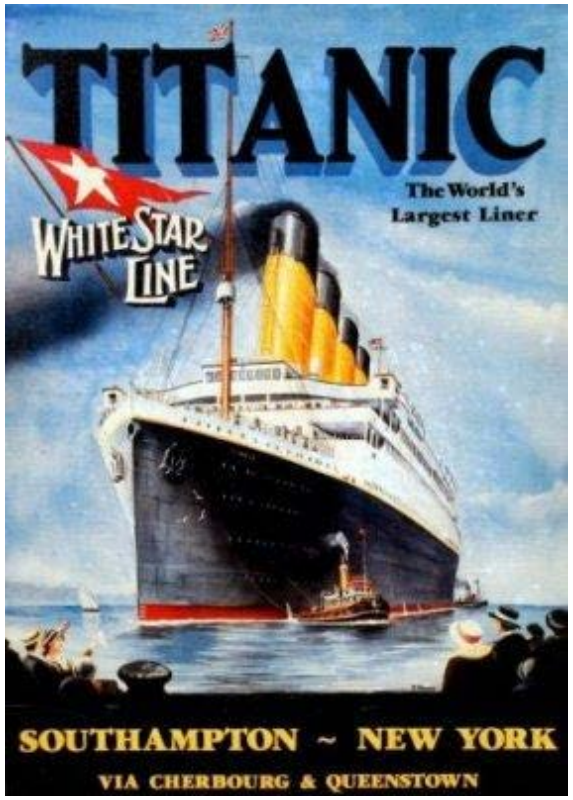
➔*Recommendations for Designing and Conducting Cold-Fill-Hold Challenge Studies for Acidified Food Products*

Ref:
NACMCF. 2010. J Food Prot 73:140-202.
Breidt et al. 2018. Food Prot. Trends 38. Sept/Oct.

Manufacturing…

Worst case scenario…

- Knowledge of product variability
- ≥ 2 independent batches
  - Ingredient and varietal variability
- pH and temperature
  - Lowest acid and highest pH
  - Type and concentration of acid defined
  - Lower temperature limit identified
- Preservatives carefully controlled
- Native microbiota accounted for
  - Enumerate native microbiota; employ an un-inoculated control
- Packaging and package atmosphere affect pathogen survival



http://blog.sfgate.com/stew/2012/04/12/historic-images-from-the-titanic-sinking/#photo-82239

Basic assumptions: math and microbiology…

- Log_{10} is used to simplify very large numbers in microbiology…

Log_{10}	Number
0	1
1	10
2	100
3	1000
4	10000
5	100000
6	1000000

5-log reduction = 100,000 fold reduction

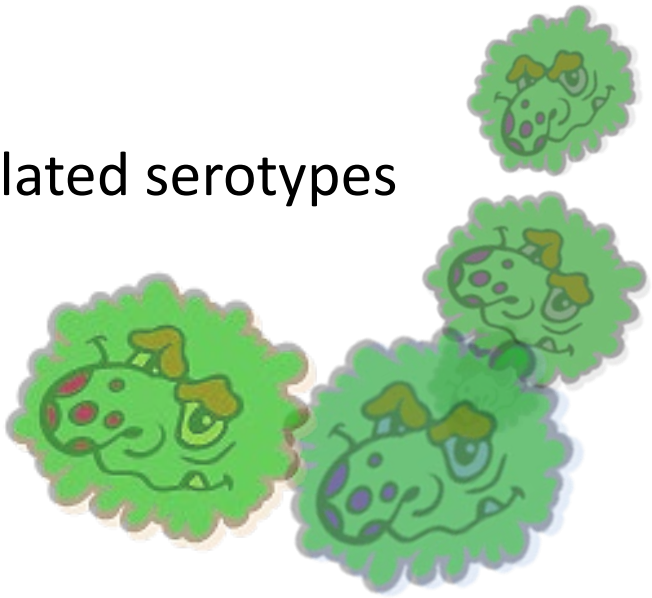
CFU/ml = Colony forming units per milliliter

Overview of Experimental Design

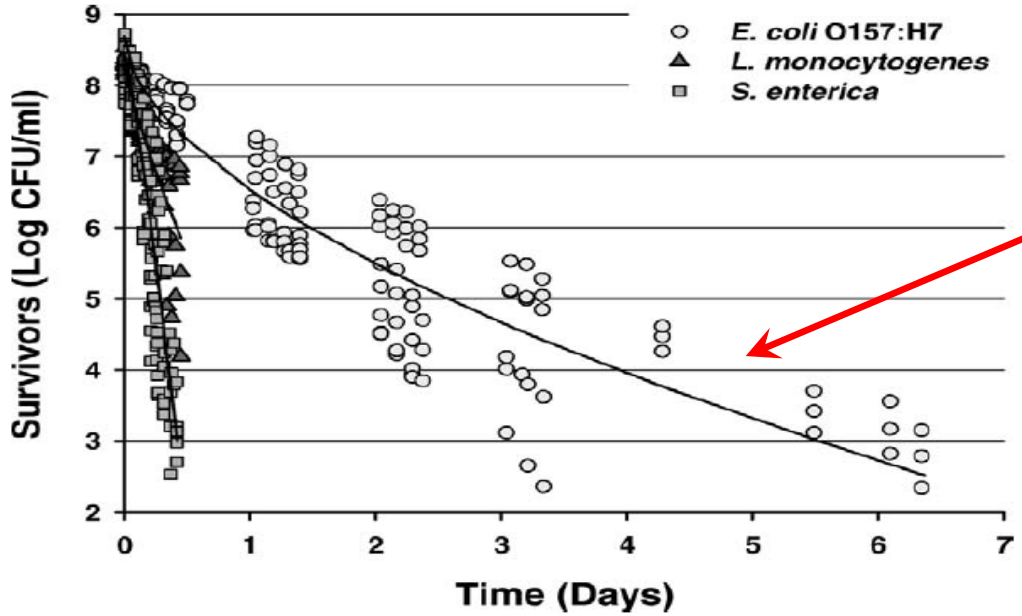
- Prepare test products
  - Spices and other ingredients may influence results
  - Independent lots of materials
- Prepare bacterial cultures
  - Acid resistance varies!
  - Independent cell cultures – avoid repeated measurements with the same cell preparation
- Prepare a sampling schedule
  - Recommended: One whole jar or container per sample time
- Analysis methods
  - Data should show an entire 5-log reduction killing curve
  - Graph all data points and regression line (or curve)

Acid resistant pathogens

- Of concern for acidified foods:
  - *Escherichia coli* O157:H7 and related serotypes
  - *Salmonella enterica* strains
  - *Listeria monocytogenes*
- *Staphylococcus aureus*
  - Salt resistant
- Refrigeration
  - *Listeria* can grow at refrigeration temperatures
- Spore-formers inhibited by pH below 4.6
  - *Clostridium botulinum*



E. coli O157:H7 is the pathogen of concern



Survival of *E. coli*

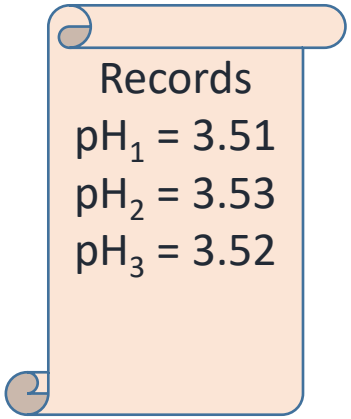
FIGURE 1. *The survival of* E. coli *O157:H7,* S. enterica, *and* L. monocytogenes *strains in acidified pickle jars at 10°C. The data for* E. coli *O157:H7 (circles),* S. enterica *(triangles), and* L. monocytogenes *(squares) show the log of the viable cell count from seven or more replicate experiments, each with a cocktail of five strains of a given species. The solid lines represent the predicted survival curves from the Weibull model.*

Ref: Breidt et al. , 2007. J Food Prot 70:2638–2641.

Environmental Variables

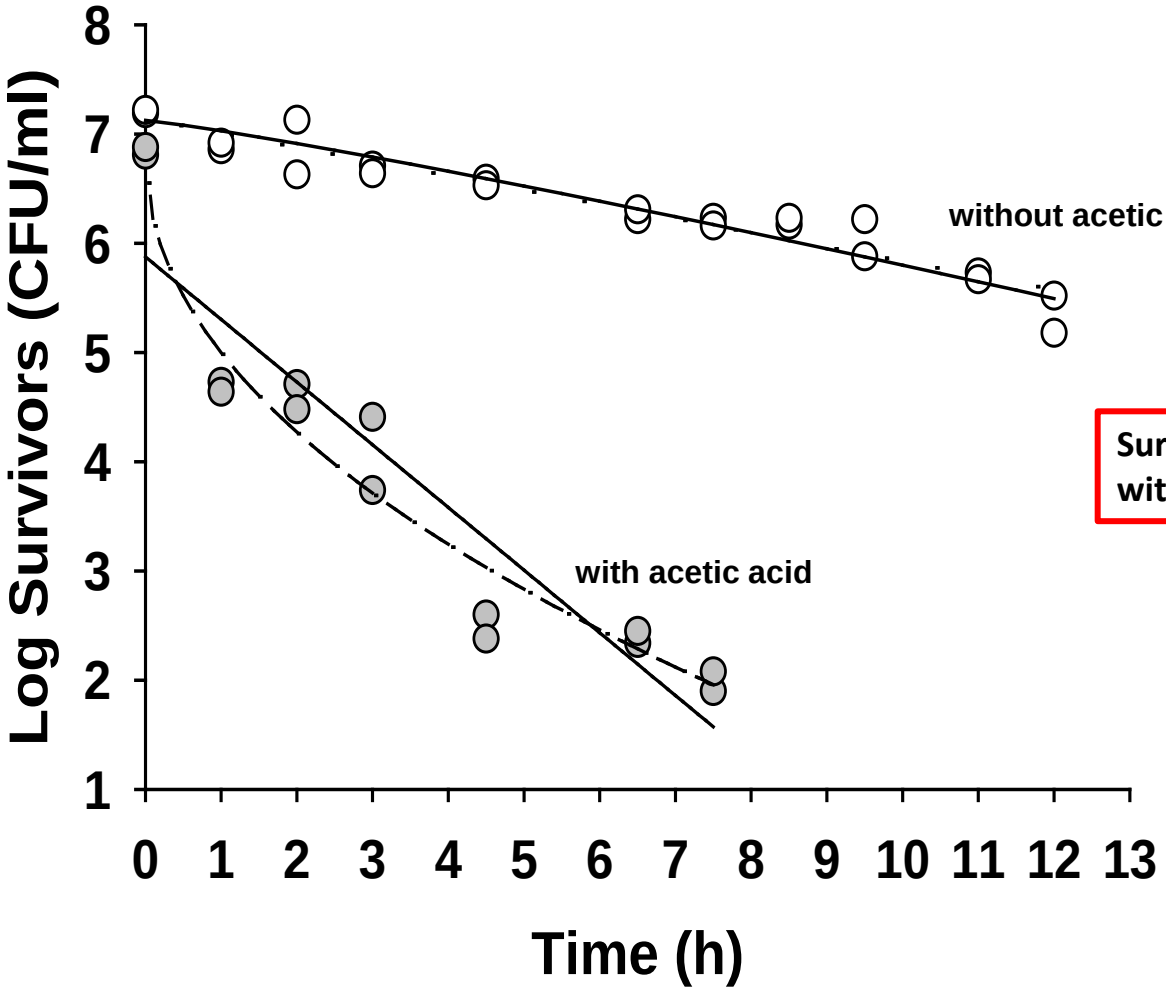
- Salt
  - Salt concentration may influence acid killing
  - Use the same salt concentration as in the product
- Temperature
  - Cold temperatures = better survival
- Oxygen
  - Dissolved oxygen can influence acid killing
  - Conduct tests in jars or packaging used for the product
- Preservatives
  - Sodium benzoate and potassium sorbate

pH is critical!



- Measurements of pH
  - Before and after challenge test
  - At each sampling time for a killing curve
- Acid type and concentration
  - Different acids have different killing effects!
  - Acid type and concentration need to be controlled and reported
  - Maximum pH and minimum acid concentration for product

Acid type and concentration matter



Survival of *E. coli* strains at pH 3.1 with or without acetic acid

Ref: Breidt et al., 2013. J Food Prot 76:1245-1249.

Example: *E. coli* O157:H7 survival at pH 3.5 and 3.8

TABLE 2. *Acid solutions and 5-log reduction times of inoculated pathogens in brined cucumbers*

Species[a]	Acid concn (%)[b]		pH	5-log reduction time (days)		R^2[c]	No. of replicates
	Acetic acid	Benzoic acid		Mean	SE		
E. coli	2.5	0	3.5	4.0	0.23	0.88	12
	2	0	3.5	11.7	0.43	0.90	11
	2	0.1	3.5	1.5	0.05	0.99	3
	1.5	0.1	3.5	3.6	0.43	0.79	6
	0[d]	0.1	3.5	14.5	0.97	0.88	3
	2.5	0	3.8	11.3	0.75	0.90	3
	2.5	0.1	3.8	3.6	0.14	0.99	3
	2	0.1	3.8	10.2	0.51	0.95	3
	1.5	0.1	3.8	13.5	1.03	0.88	3
Salmonella	2.5	0	3.5	1.5	0.11	0.91	3
	2.5	0.1	3.8	1.6	0.08	0.97	3
	1.5	0.1	3.5	0.6	0.03	0.97	3
L. monocytogenes	2.5	0	3.5	0.6	0.03	0.95	3
	2.5	0.1	3.8	1.5	0.24	0.79	3
	1.5	0.1	3.5	0.3	0.01	0.98	3

[a] Each species cocktail contained five strains.
[b] All acid concentrations were ±0.1% of the indicated target concentrations.
[c] Value for the linear regression used to calculate the 5-log reduction time.
[d] This acid solution contained 0.5% citric acid buffer.

Acid concentration matters!

Ref: Breidt et al., 2013. J Food Prot 76:1245-1249.

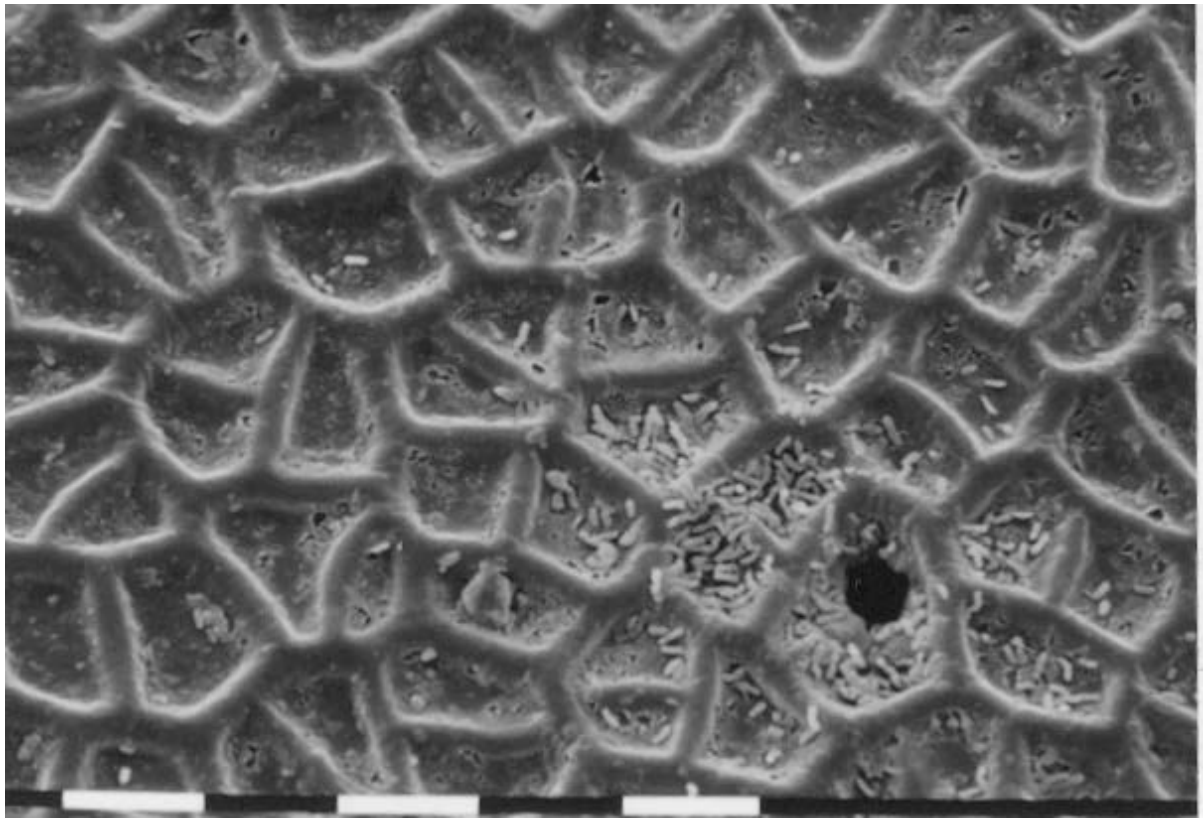
Cold fill acids & *E. coli* O157:H7 log reduction

Acid	Concentration for 5-log reduction (mM)
Malic	547.00 [e] +/- 128.00
Acetic	377.00 +/- 21.00
D-lactic	140.00 +/- 7.00
L-lactic	124.00 +/- 3.00
Fumaric	24.11 +/- 3.28
Sorbic	6.59 +/- 0.54
Benzoic	6.47 +/- 0.38
Sulfite	1.27 +/- 0.12

➔The TYPE of acid in the formulation matters!

Ref: Lu et al., 2011. J Food Prot 6:893-898.

Bacteria in the interior!



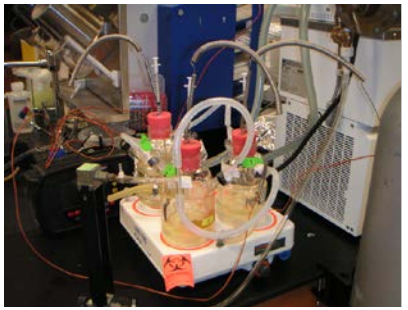
Enterobacter aerogenes on the surface of a cucumber
Bar = 10 microns

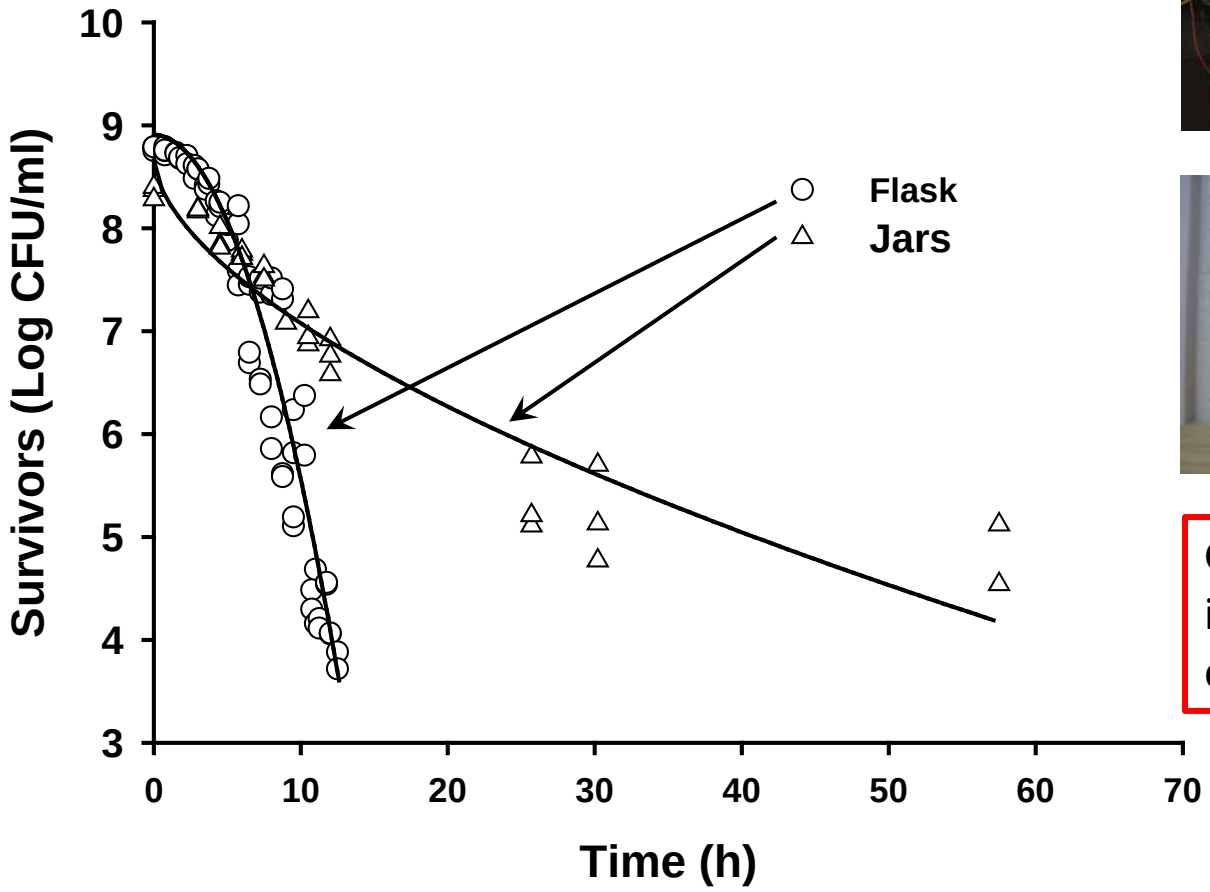
➔ Acid and pH equilibration may be important

Ref: Reina et al., 2002. J Food Prot 65: 1881-1887

Packaging:

The importance of doing studies in the appropriate packaging



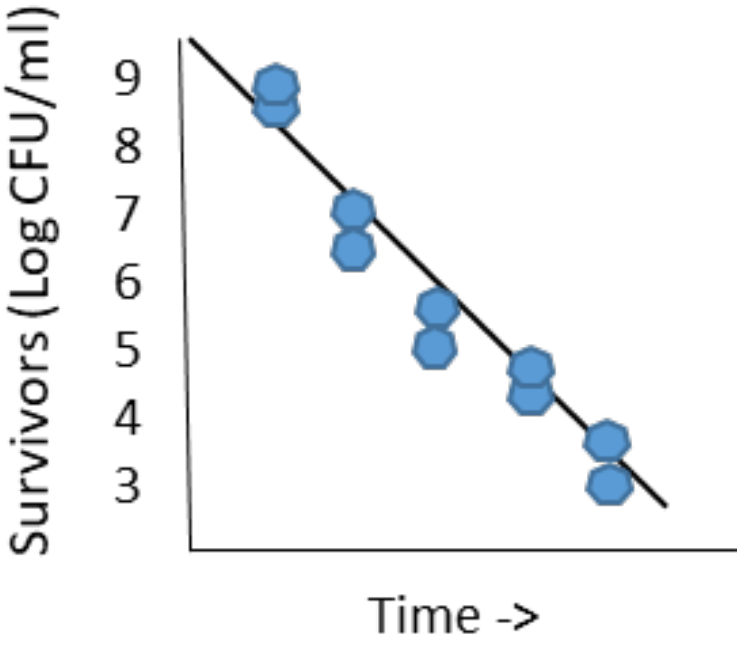


Greater survival in an anaerobic environment.

Ref: Kreske et al., 2008. J Food Prot 71:2404-2409.

Challenge study – bacterial cells

- Static growth in glucose helps induce acid resistance
  - *E. coli* O157:H7 cocktail
  - Stationary phase cells are acid resistant
  - Multiple strains
  - Record initial numbers: before and after inoculation
- Record cell counts at least 4 different times
  - Showing at least a 100,000 fold (5-log) reduction
- Use non-selective media if possible
- Graphical representation facilitates interpretation
  - Log scale (base 10)
    - Log CFU/ml *vs.* Time



Measurement of 5-log reduction

- 5-log reduction
  - 100,000 fold reduction in CFU/ml
- Plating limit of detection
  - Spread plate method: typically 100 µl plated
    - Count 20-200 colonies
    - Limit of detection apx. 200 CFU/ml in sample
  - Spiral plating: typically 50 µl plated
    - Spiral counting method
    - Limit of detection apx. 400 CFU/ml in sample
- Starting cell count
  - CFU/ml count above detection limit (2 to 4 x 10^2)
  - Greater than 2 to 4 x 10^7, typically 10^8 CFU/ml
- Use Log_{10} numbers!

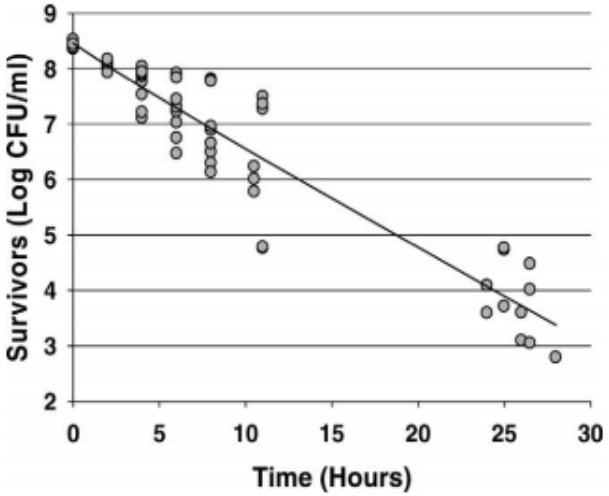
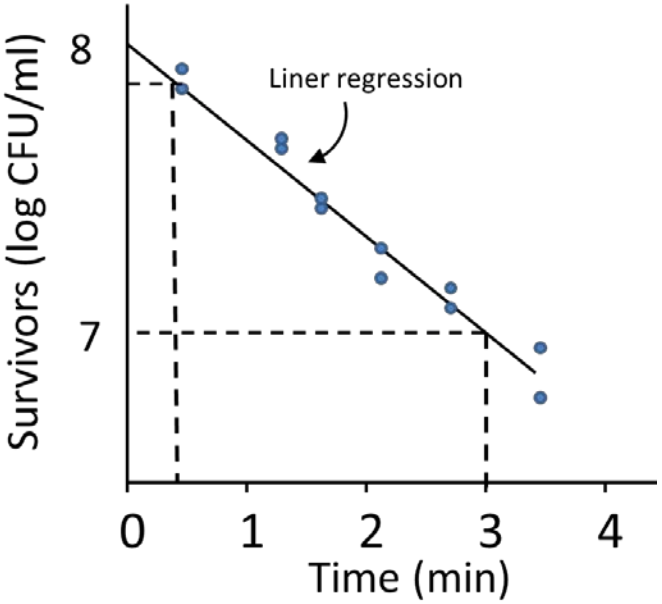


FIGURE 2. *The survival of* E. coli *O157:H7 in acidified pickle jars at 25°C. The data for* E. coli *O157:H7 (circles) show the log of the viable cell count for nine independent replications with a five strain cocktail. The solid line represents the predicted survival curve from the Weibull model.*

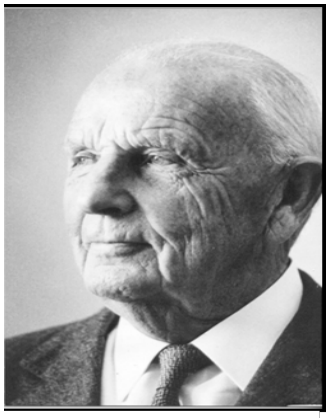
Breidt et al., 2007. J Food Prot 70(11):2638-2641.

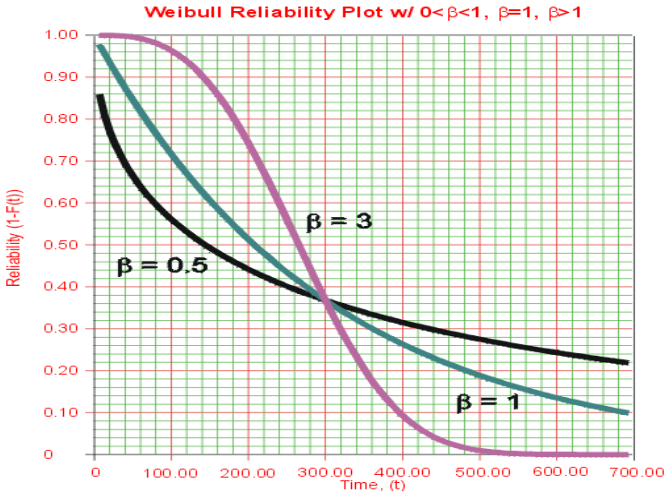
Data Analysis Models

5-log reduction times



Rocket science!


Wallodi Weibull 1887-1979
Photo by Sam C. Saunders



Linear: Log_{10} CFU/ml = $m\tau + b$

Linear 5-log Reduction Time = $5(-1/m)$ (5 times D value)

Weibull: Log_{10} CFU/ml = $N_o - (1/\ln[10])(\tau/\alpha)^{1/\beta}$

Weibull 5-log Reduction Time = $\alpha(-\ln[(10^{-5}])^{\beta}$

Statistically speaking…

- Repeated measures vs. independent replication
  - True independent replication is needed
    - Cells grown independently, different lots of product
  - Using the same cells or lots is a repeated measure of the same thing, *not independent replication*
- Regression and goodness-of-fit
  - Use established linear or non-linear models
  - Fitting algorithms are available in Excel, or statistical analysis software
- Use log numbers for statistics!
  - Due to increase in variance as numbers get large…
- Error terms on predicted parameters
  - Variation is expected, so estimated log reduction will vary too!
  - May require the help of a statistician

Summary of methods

1. Cocktails of independently grown cells
   - Static growth in media with 1% glucose to induce acid resistance
   - Use 3-5 strains
2. Inoculum should be 1% of volume or less
   - Well mixed into product
   - Sufficient cells to allow full 5-log reduction
   - One independently grown cocktail for each rep or set of containers
3. At least TWO jars or packages per sampling time
   - Independently sourced ingredients with different lots!
   - Open and sample, don't reuse…
4. Environmental variables are CRITICAL
   - Temperature, oxygen, and pH
   - Cold temperature can influence survival, 50^{o}F is recommended
   - Sealed jars
   - pH of the product: highest pH lowest acid concentration!
5. Data presentation
   - Graphic representation of data, use log numbers for analysis
6. Records of experimental procedures and data

Exactly what was done…

ALL DATA GOES HERE…

Challenge study report identifies…..

- Formulation
  - Acid type and concentration
  - Other ingredients, including preservatives
- Manufacturing
  - Maximum pH at the filler
  - Minimum hold time and temperature
  - Container/closure/atmosphere

➔Factors that ensure a safe, shelf-stable product

➔Process authority authors report

Scheduled Process
Kitchen Sink BBQ Sauce

Sheza Star
Picklin' Princess, LLC
1234 Galaxy Lane
Anywhere, WI 54321

Critical Factors:
- Max. equilibrium pH 4.0, target ≤3.6
- Container: 16-oz (glass); 1-piece metal CT lid with button top
- Headspace: target ½"; maximum ¾"
- Fill temperature: 180°F (minimum)
- Inverted hot-fill-hold: 180°F (minimum) for 2 minutes (minimum)
- Seal integrity: button down on top indicates vacuum

Authorized supervisor: Sheza Star (Better Process Control School-Acidified, 2017)
Commercial pH test results (2 batches): 3.46, 3.52

Ingredient	Prepared Weight (oz).
Tomato Paste*	365
Water	300
Ketchup (Hunts)*	216
Apple Cider Vinegar, 5% acetic acid	83
Mustard (French's)*	62
Onions, peeled, sliced ¼" max.	60
Green peppers, topped, seeded, chopped ½" max.	54
Salt	34
Frank's Hot Sauce*	4
Worcestershire Sauce*	2.50
Garlic powder	0.7

All ingredients must be listed on the label in decreasing order by weight, all sub-ingredients must be listed. e.g.: TOMATO PASTE, WATER, KETCHUP (tomato concentrate, high fructose corn syrup, vinegar, corn syrup, salt, less than 2% of spice, onion powder, natural flavors), VINEGAR, MUSTARD (vinegar, mustard powder, EGGS, salt, spices, FD&C#2, FD&C#6), ONIONS, GREEN PEPPER, SALT, HOT SAUCE (pepper, vinegar, salt), WORCESTERSHIRE SAUCE (vinegar, molasses, water, sugar, onions, anchovies, salt, garlic, cloves, tamarind extract, natural flavorings, chili pepper extract), GARLIC POWDER. CONTAINS: EGGS, ANCHOVIES

PROCEDURE:

1) Clean containers and closures.
2) Prepare and weigh ingredients.
3) Combine all ingredients in a large kettle and heat to a boil (210-212°F), hold for 5 minutes.
4) Blend with an immersion blender until smooth.
5) Fill containers with hot sauce. Apply lid. [Sample set aside for pH testing.]
6) Immediately invert, and hold 180°F or higher for at least 2 minutes. Turn container right side up and allow to air cool. Hot time and temperature are confirmed on one container per batch and the results recorded.
7) Check pH on room temperature sample and within 24 hours to ensure that pH is under 4.0 before product is shipped. pH is measured directly, no sample preparation needed. pH will be measured with a properly calibrated pH meter or litmus paper in the proper range.
8) Record all critical factors.

Any change to recipe, process, container or closure must be reviewed by a Process Authority. Label jars with product name, legal name of business, business address (street address, city/state/zip), ingredient statement, net weight (oz and grams), and lot code. Lot code must include establishment, product, year, day and period when packed. Container closure must be evaluated and records kept. *Pre-packaging and post-packaging heating steps are sufficient to ensure minimum time/ temperature process lethality as defined by* F. Breidt, K.P. Sandeep, and F.M. Arritt. 2010. Use of linear models for thermal processing of acidified foods. Food Protection Trends 30:268-272. Scheduled process authorized for Sheza Star only at Picklin' Princess, 1234 Galaxy Lane, Anywhere, WI effective xx XXXX 2017.

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←Critical Factors

-pH
-container type and size
-closure
-headspace
-fill temperature (minimum)
-cold-fill-hold parameters
-seal integrity

←Formulation

←Processing Steps

-Times and temperatures 'in the kettle'
-Cold fill temperature, hold time
-pH sampling

Wrap up…

https://journeysinclassicfilm.com/2016/10/07/the-mummy-1932/

- Process authority authors report
- Include adequate information to facilitate regulatory review
- Report contains:
  - Introduction, design, methods, data, graphs, summary, conclusion
  - 5-log reduction estimate
- Processor complies with FDA process filing requirements:
  - FDA form 2541
  - FDA form 2541e (for each product/container)

➔Public health is protected.

References

- Breidt et al. 2007. J Food Prot. 70 (11): 2638–2641
- Breidt et al. 2013. J Food Prot 76(7): 1245-1249.
- Breidt et al. 2018. Food Prot. Trends *in press* (Sept/October 2018)
- Kreske et al. 2008. J Food Prot 71(12): 2404-2409.
- Lu et al. 2011. J Food Prot 6: 893-898.
- NACMCF. 2010. J Food Prot 73: 140-202.
- Reina et al. 2002. J Food Prot 65(12): 1881-1887

- Pickle bibilography URL (Google “pickle bibliography”)
  https://fbns.ncsu.edu/USDAARS/html/Fflbiblio1.htm
- 21 CFR 114 URL
  http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=114

The Microbial World…

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