INGREDIENT IMPACT ON SAFETY

Robin Peterson

National Accounts Manager
Corbion Purac

Factors which influence growth of pathogens

A review of the parameters which influence safety

- Moisture
  - Ionic strength
  - Aw
- pH
- Temperature
- Species: The “X” Factor

Effect of Various Ingredients

- Smoke
- Carrageenan
- Starch
- Nitrates/ites
- Antimicrobials

©

Moisture

- Ionic strength and Aw are a function of moisture and concentration of dissolved solids (salts, sugars, proteins)
- Manage moisture by keeping it physically compartmentalized or by managing migration (batter breaded items)
- High moisture = lower Ionic Strength = Higher Aw = higher bacterial growth
- Free moisture = purge…usually bad unless it is a vehicle for inhibitor (LAE)

©

Corbion

Ionic Strength

Ionic strength (μ)is a measure of in a solution:

1. The number of ions (using a molar concentration)
2. Their charges

Ions produce an osmotic stress on micro-organisms

Some specific ions also promote or inhibit growth (Na+)

Multiple charge ingredients have larger effect (CA ++, Phos ++, +++)

Ionic Strength is similar in concept to water activity, but does not capture the non-charged dissolved compounds (such as sugars) that can inhibit microorganisms

$$\mu = \frac{1}{2}\sum_{1}^{n} c_i z_i^2$$

©

Water Activity

Definition:

- Ratio of vapor pressure in a system vs. vapor pressure of pure water at same temperature

Range: 0-1

Influenced by pH

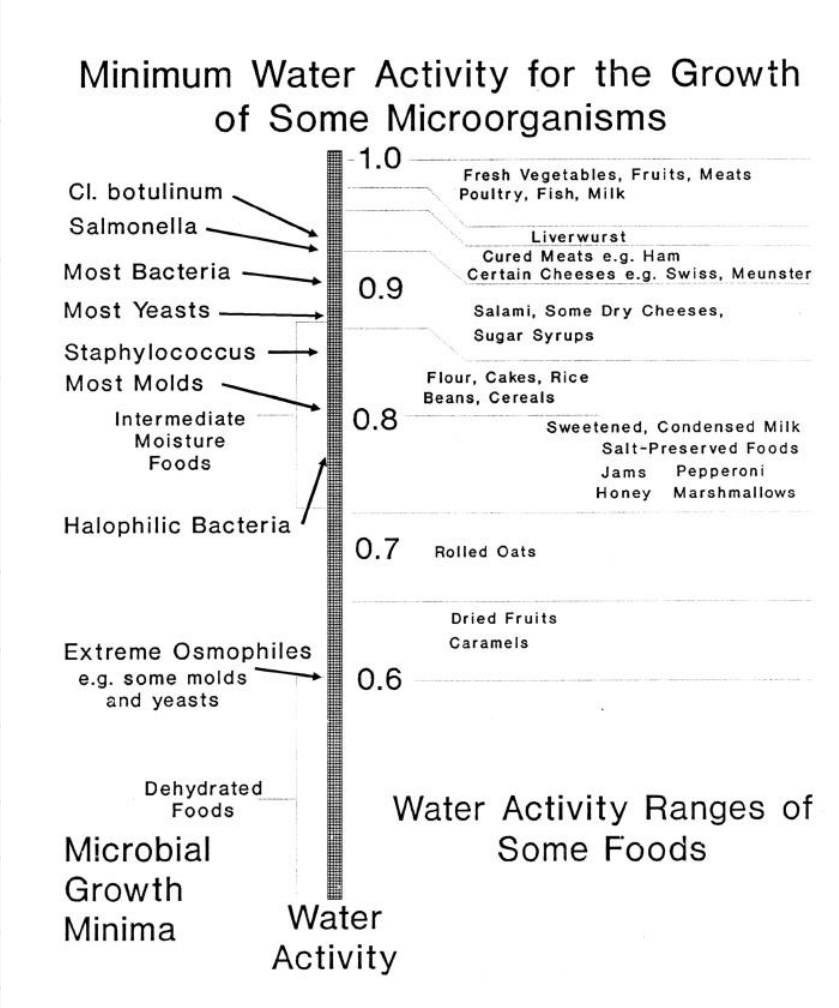
Influences bacteria's reproduction, metabolic activity & ability to survive

At Aw = 1 freezing point is 32 and boil is 212

- .98 or higher…supports growth
- .94 not likely to support growth *Salmonella, Clostridia*
- .92- *LM* wont grow below this Aw
- .8 Mold will grow, not bacteria

©

Water Activity- levels needed for various bacteria



©

Corbion

Water Activity- Micro

TABLE 1. Minimal a_w for multiplication of micro-organisms associated with meat and meat products

a_w	Bacteria	Yeasts	Moulds
0·98	*Clostridium* (1), *Pseudomonas**	—	—
0·97	*Clostridium* (2)	—	—
0·96	*Flavobacterium, Klebsiella, Lactobacillus,* Proteus,* Pseudomonas,* Shigella*	—	—
0·95	*Alcaligenes, Bacillus, Citrobacter, Clostridium* (3), *Enterobacter, Escherichia, Proteus, Pseudomonas, Salmonella, Serratia, Vibrio*	—	—
0·94	*Lactobacillus, Microbacterium, Pediococcus, Streptococcus**	—	—
0·93	*Lactobacillus,* Streptococcus, Vibrio**	—	*Rhizopus, Mucor*
0·92	—	*Rhodotorula, Pichia*	—
0·91	*Corynebacterium, Staphylococcus* (4), *Streptococcus**	—	—
0·90	*Micrococcus, Pediococcus*	*Saccharomyces†, Hansenula*	—
0·88	—	*Candida, Torulopsis*	*Cladosporium*
0·87	—	*Debaryomyces†*	—
0·86	*Staphylococcus* (5)	—	—
0·85	—	—	*Penicillium†*
0·75	*Halophilic bacteria*	—	—
0·65	—	—	*Aspergillus†*

The Significance of Water Activity for Micro-organisms in Meats

L. LEISTNER AND W. RÖDEL

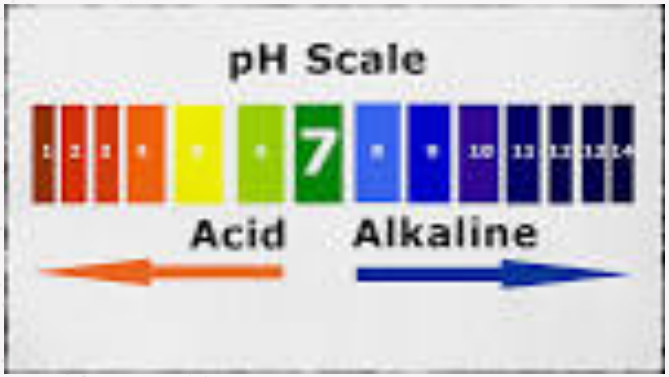
Bundesanstalt für Fleischforschung, Kulmbach, Bundesrepublik Deutschland

Minimum Aw needed for:
LM- .92
Salmolla and ecoli - .95
Staph Aureus -.86

©

pH and influence on pathogen growth

- Each organisms has a preferred pH and become stressed by pH extremes
- Pathogens of concern don't survive well below pH 5.2
- Processed meats typically fall within pH 5.8-6.4
- (fermented/dried pH 4.6-5.3)
- pH comes into play in discussions w /organic acids
- The lower the meat pH the more effective the antimicrobial
- The lower the meat pH the more issues w/ yield/purge/texture
- It's a balance



©

Species influence

- We know a species difference exists
- LM will grow faster in a Turkey product vs. Roast Beef given the same parameters of pH, moisture, Aw,
- Turkey is better matrix for Lm growth then beef or pork- why no one knows
- Focus has been on finishing solution even w/o ID the problem specifically w each species

Research opportunity?

©

Ingredients and their impact on safety

©

Salt is Salt is Salt?

Acts as an antimicrobial, among other contributions

1. Increases ionic strength
2. Decreases Aw

On a weight basis NaCl has a greater effect then KCL

- Na is a smaller molecule

On a weight basis Sea salt = regular salt on level of sodium

- Impurities do not influence in a meaningful way

On a Volume basis (Culinary) Sea Salt has less sodium: larger crystals

Other functions of salt:

1. Causes protein swelling and unfolding of the muscle proteins
2. Makes more sites available for water binding
3. Aids in texture

©

Natural Smoke: Antimicrobial Properties

- Disrupts cellular membrane- data shows both cidality and stasis
- Effective antimicrobial agent against an array of bacterial pathogens such as L. mono, Salmonella, spp., pathogenic E. coli and Staphylococcus, spp.
- Being used more frequently in preserving protein-based food
- May satisfy consumer demand for more all-natural foods while maintaining safety
- Preservative effect is achieved by Carbonyls (aldehydes), carboxylic acids (organic acids) and phenols (smoke flavor compounds)

Lingbeck, J.M., Cordero, P, O'Bryan, C.A., Johnson, M.G., Ricke, S.C., Crandall, P.C. (2014) Functionality of liquid smoke as an all-natural antimicrobial in food preservation. Meat Science 97, 197-206.

©

Wood Smoke: Components

Traditional Wood Smoke:

(1,200 different compounds)

Vaporous Phase

- Organic acids
- Alcohols
- Smoke flavor compounds
- Carbonyls (browning compounds)
- Organic vapors
- Carbon monoxide

Particulate Phase

(Contains Polycyclic Aromatic Hyrdocarbons (PAH's))

- Fly ash
- Tars and resins

Condensed Natural Smoke: *Produced through a controlled pyrolysis of hardwood sawdust*

(<750 different compounds)

Vaporous Phase

Organic Acids

Smoke flavor compounds

Carbonyls (browning compounds)

No Particulate Phase

©

Condensed Natural Smoke Composition

Organic Acids (primary: acetic, proprionic, formic):

- Skin formation via coagulation of surface proteins
- Cure reaction acceleration
- Preservation effect > antimicrobial
- Flavor: tartness

Smoke Flavor Compounds: (Phenolics)

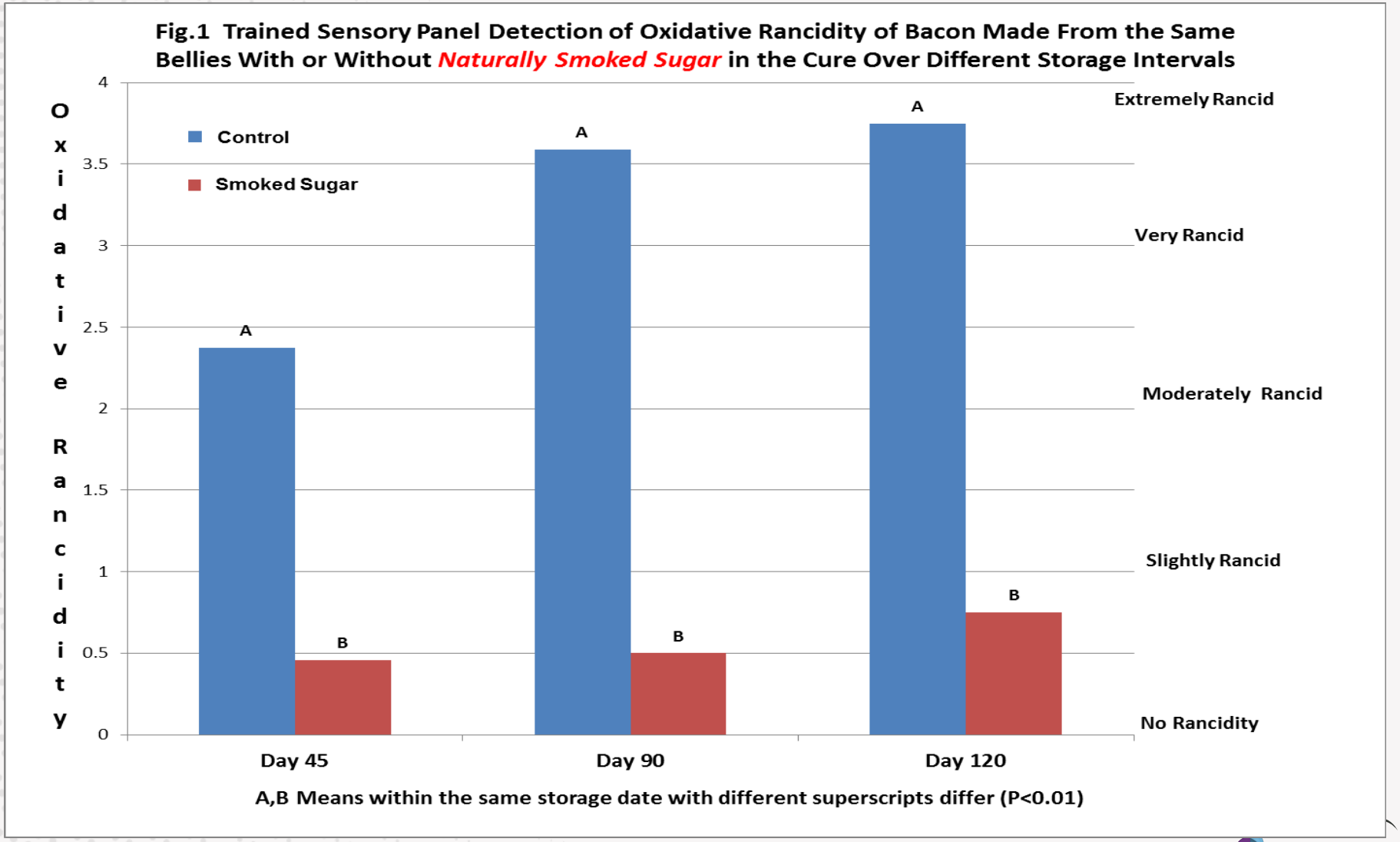
- Flavor and aromatics
- Antioxidant properties
- Color > oil-soluble provides color to fat

Carbonyls:

- Maillard reaction: carbonyl-amine groups
- Crosslink proteins: skin formation/texture
- Antimicrobial properties: preservation effect

©

Naturally occurring compounds present in wood smoke are very effective antioxidants (in particular, syringol, eugenol, iso-euginol, and catechol)



©

Corbion

Antimicrobial Properties

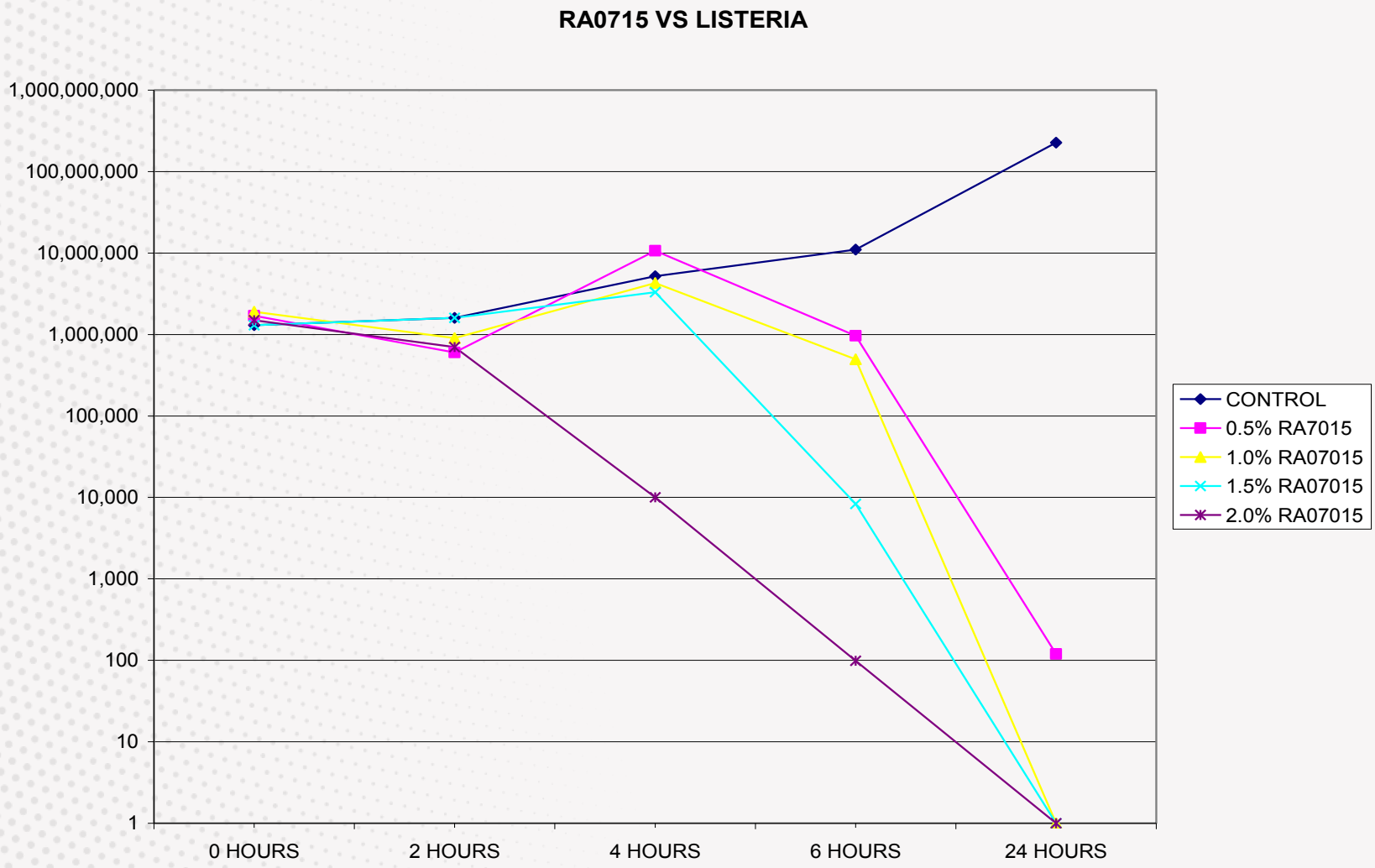
Strongest contributing Carbonyls:

- Glycoaldehyde
- Glyoxal
- Acetol
- Glyceraldehyde
- Methylglyoxal
- Acrolein

©

Natural Concentrated smoke flavor vs LM

Effect of natural smoke against LM in a broth culture- Kills



©

Corbion

Phosphates

Increase ionic strength

On a weight basis phos contributes to same level of benefit towards increasing ionic strength as does salt…however

Often raises product pH

which decreases effectiveness of organic acid antimicrobials

How phosphates function:

Reduce Aw by allowing protein to bind water more efficiently by:

- separating actomyosin into actin and myosin
- Chelating divalent cations
- Complex metal ions that reduce water holding capacity
- Mimic function of ATP in live muscle- energy transport
- Raise pH to improve water holding capacity
- Used to improve water binding

©

Nitrates- Nitrites & Effect on pathogens

Discovered as anti clostridial

Functions by binding to iron and sulfur in the microorganism,

Prevents toxin formation

Not clear how it functions on Lm

Influences types of micro species which grow, especially under anaerobic conditions can effect shelf life

- Inhibits gram negatives more easily, resulting in gram + growth
- Grow lactics= sour, slime, gas

©

Carrageenan and Starches

Little to no influence on pathogen growth

No/minimal influence on Aw, pH or ionic strength

Purge reduction is primary effect = less transportation for bacteria

Combined Functions:

- Bind water, reduce purge
- Improve texture and mouthfeel
- Increase Water Holding Capacity
- Improves Emulsion Stability
- Improve sliceability

©

Corbion

Seasonings & Sugars

Oregano, Cinnamon, Rosemary, Sage: All show some level of inhibition

- **no safety credit taken,**
- **no change in Aw or ionic strength**
- **Minor increase in water binding**

Sugars: usually work against safety and shelf life

- **provide energy source for spoilage bugs and LM**

©

Types of Antimicrobials

Organic Acids

Antimycotics

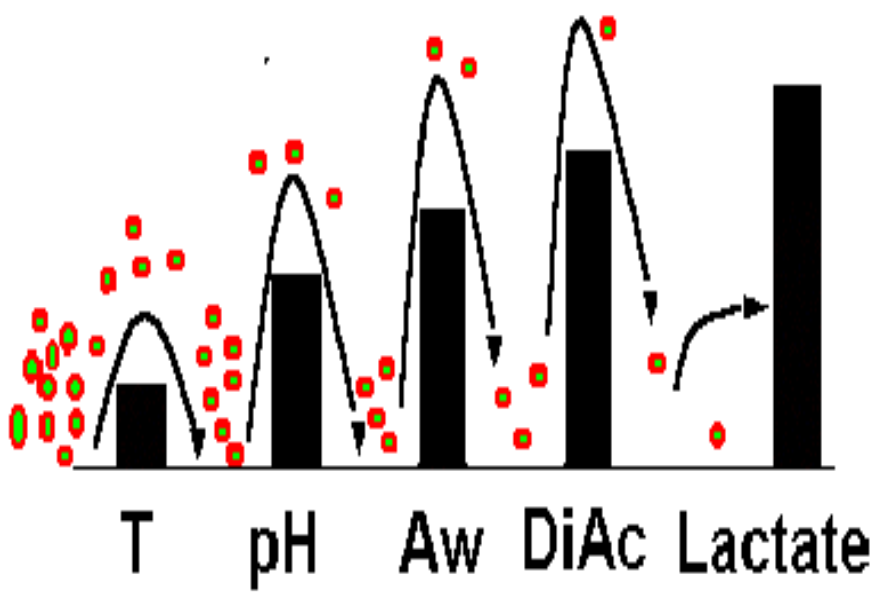
Bacteriocins

Fatty Acids

Bacteriophages (virus)

©

Food Preservation Hurdle Technology



- Each hurdle affects the growth of micro organisms
- More hurdles means more restrictions for bacteria growth

©

Corbion

Multiple Interventions to Protect Meats

1. Use of lactic or other acids or chemicals to spray on carcass during slaughter
2. Use of similar sprays on primals or sub primals
3. Use of antimicrobial ingredients inside product formulations- raw or cooked
4. Use of surface treatments to protect against the growth of listeria and other pathogens
5. Equipment- High pressure, Post Pasteurization, Irradiation

Corbion

Antimicrobial Categories

Bactericidal

- Kills bacterial cells
- Commonly applied topically
- Post lethality microbial reduction

Bacteriostatic

- Inhibits bacterial cells
- Commonly formulated as an ingredient
- Microbial suppressor

Corbion

Organic Acids vs Salts

Bactericidal:

Organic Acids- lower pH

- Lactic acid
- Acetic acid
- Citric acid
- Propionic acid
- Benzoic acid
- Sorbic acid
- Propionic

Bacteriostatic:

Organic Acid Salts- minor pH change

- Lactate
- (Di)Acetate
- Citrate
- Propionate
- Benzoate
- Sorbate
- Propionate

Corbion

Common Organic Acids

Lactic- lactates- Sodium & Potassium

- Present in meat muscle, widely used

Di-acetate/Acetate/ Vinegar

- hydrophillic, good inhibitor, widely used

Cultured dairy or dextrose products

- Gaining traction for use due to clean labeling

Citric- citrates

- Hydrophilic, some use against lm

Corbion

Organic Acids: Factors Influencing Efficacy

- **pKa value: level of un-dissociated acid ions**
  - Ion w/o a charge available to cross the microbial cell membrane
- **Lipophilic vs. hydrophilic nature**
  - Attracted to or repulsed from micro membrane
- **MIC value**
  - Minimum Inhibitory Concentration
- **Flavor**
- **Taste**
- **Safety of consumption**

Corbion

How Organic Acid Salts and Acids inhibit growth & Kill

Salts for Stasis:

- Un-dissociated acid ions transport thru the cell membrane
- Disrupts bacterial cell pH gradient
- Organic acid salts goes into cell, interrupts internal mechanism by requiring the cell spend energy to transport the salt out, hence reducing energy spent on growth

Acids for Kill:

- Used in carcass decontamination- combined w/ hot water
- Acid shocking: denatures proteins on cell membrane, effects pH gradient

Corbion

Vinegar, Acetate and Di-acetate

- **Vinegar:**
  - Acetic Acid solution
  - Lowers the pH
  - Typical use 1.5-2.5% liquid or .8% powder
  - Potent
  - Pungent odor
  - Hydroscopic when dried
  - Fastest growing antimicrobial category
- **Acetate:**
  - Salt of acetic acid
  - Does NOT lower pH
  - Use up to 0.25%
  - Potent
  - Potential odor
  - Hygroscopic
- **DiAcetate**
  - Dissociates to 60% sodium acetate and 40% acetic acid
  - Lowers pH
  - Use up to 0.25%
  - Potent
  - Hygroscopic

Corbion

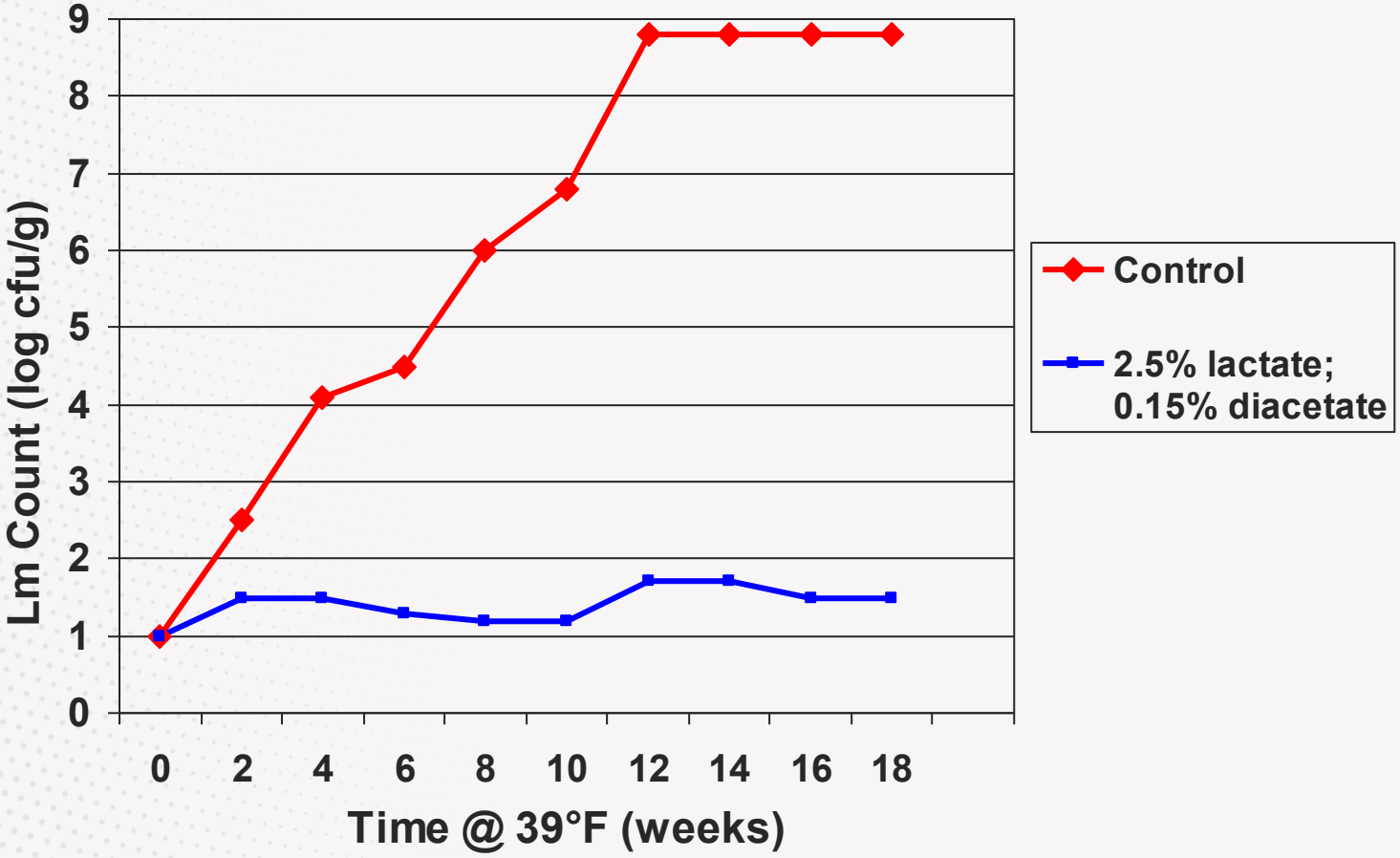
Ferments/Cultured Dextrose or sugars

- Clean label but in most cases not natural
- Naturally fermented sugars using specific food cultures:
  - produce a range of organic acid salts and fermentation products
- Contain a mixture of organic acid salts, peptides, sugars
- Light-medium brown solutions
- Can be blended with vinegar to aid in Listeria control
- Some may enhances meat flavors- meaty, brothy, savory
- Some may be good for sodium reduced products

- Danisco – MicroGARD™, NovaGARD™
- Kemin - SHIELD®
- Kerry – Alta®
- Corbion - Verdad®

©

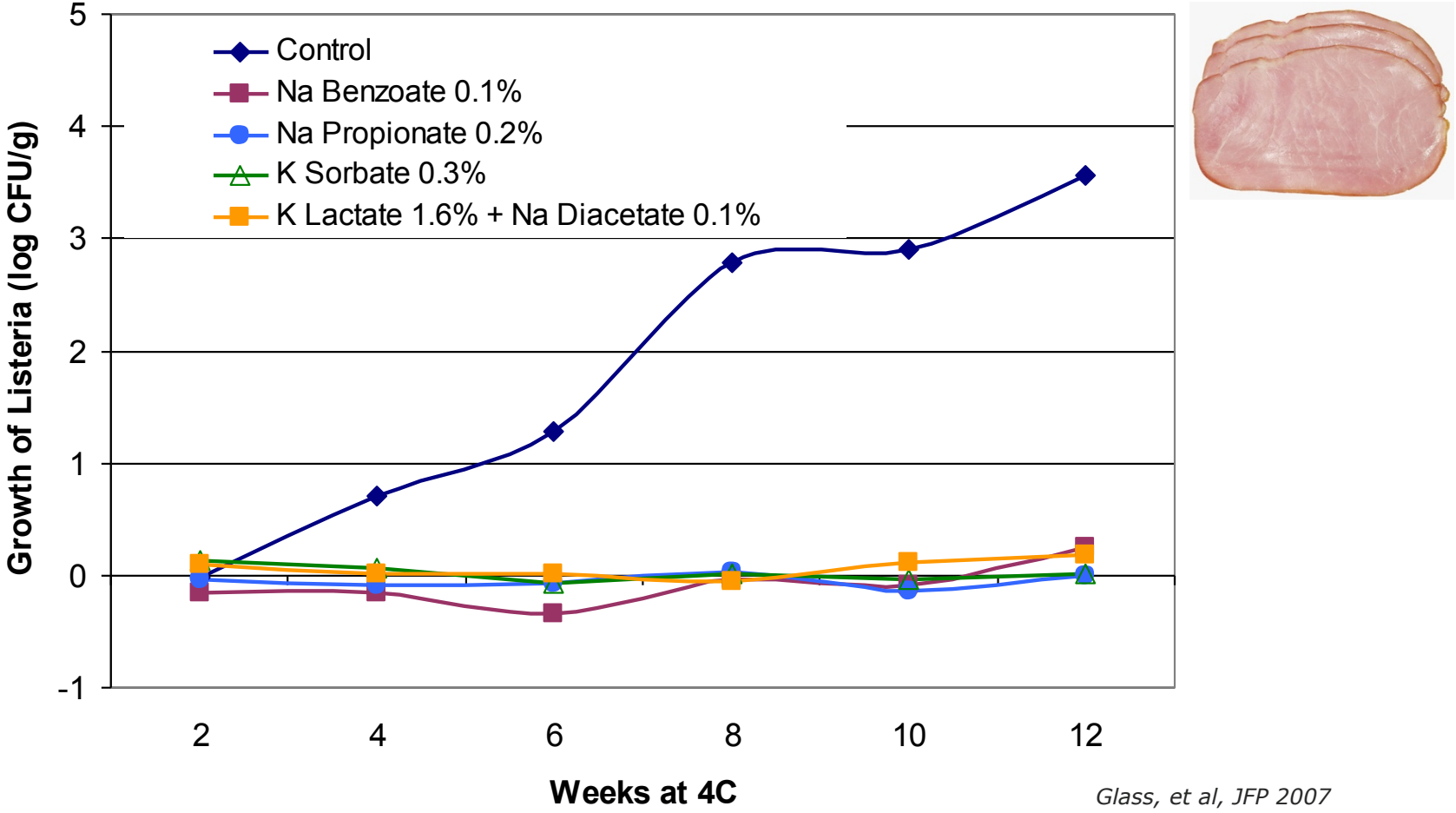
Effect of Potassium Lactate and Diacetate on *Listeria* in Ham



(Seman *et al*, J.of Food Protection, 2002)

Corbion

Control of *Listeria* on Cured Ham stored at 4°C (2.2% NaCl whole; 156 ppm nitrite, 75% moisture)



Glass, et al, JFP 2007

Corbion
purac

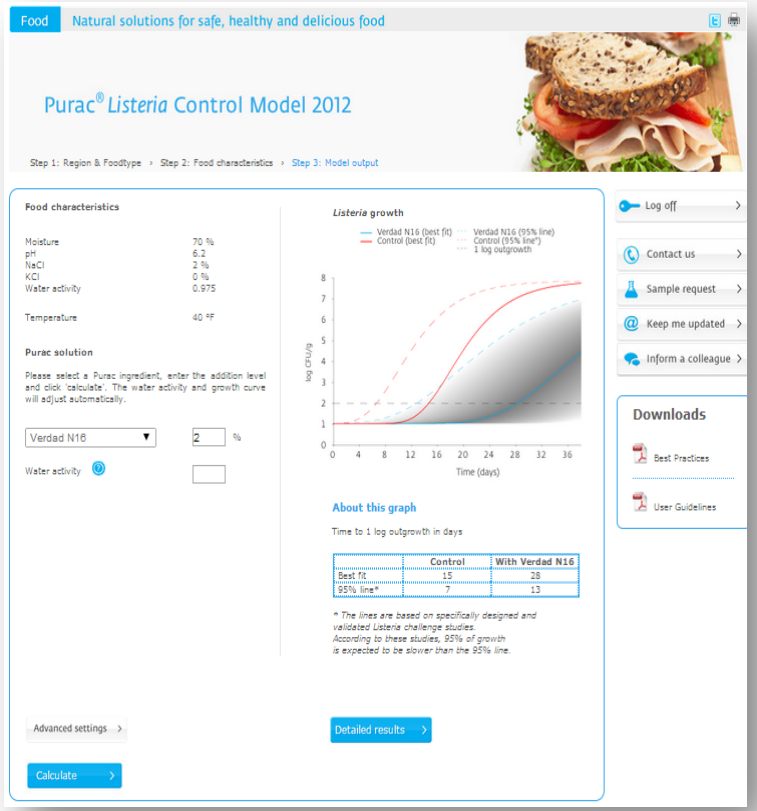
LISTERIA CONTROL MODEL 2012

Effect of organic acids on LM growth

Corbion *Listeria* control model

Originally developed by Oscar Mayer/Kraft in 2001 (Seman et al, 2002)

1. Use knowledge of over a decade of *Listeria* studies to accurately predict *Listeria*
2. Predict *Listeria* growth and safety levels using a specific set of food characteristics
3. Calculates level of Corbion ingredient needed to achieve targeted safety levels



Corbion

Model Development

Gamma Concept

The 'growth rate' and the 'adaptation time' (lag time) depend on

- ✓ pH
- ✓ the type of organism
- ✓ its environment
- ✓ the change in environment (lag time)

A number of key food characteristics can be used to predict its growth:

- ✓ pH
- ✓ (storage) temperature
- ✓ moisture content
- ✓ water activity (= effective water concentration)
- ✓ concentration of components that influence the growth, such as salts and preservatives

Listeria Control Model 2012

New Features & Functionalities

Over 2,600 growth curves have been used to fit and validate the 2012 model

(new) Functionalities	
Water activity	pH
NaCl	KCl
Temperature	Nitrite (levels)
Lactate	Acetate
Propionate	Newest Corbion products

New features

- Available online → everything in one place and easier to update
- Output: best fit and 95% confidence lines (vs. 90/95% lines in 2007 model)
- Automatically generated PDF report of results
- Simulate sodium reduction
- Adjustable nitrite levels

Listeria Control Model 2012

Ranges of parameters

Parameter	Range
Water activity	0.96-0.99
pH	4.7-6.8
Lactate	0-3%
Acetate	0-1.5%
Propionate	0-0.45%
NaCl	0-4.7%
KCl	0-2%
Sodium nitrite (ingoing; based on total formulation)	0-200ppm
Temperature	4-10°C (39-50°F)

Listeria Control Model 2012

Detailed Results

Results available in 4 steps

Region & Food Type – Model Input – Corbion Solution – Detailed Results

Detailed results are directly downloadable

- 2 page report
- Details of growth
- Summary of input
- Easy to print/save

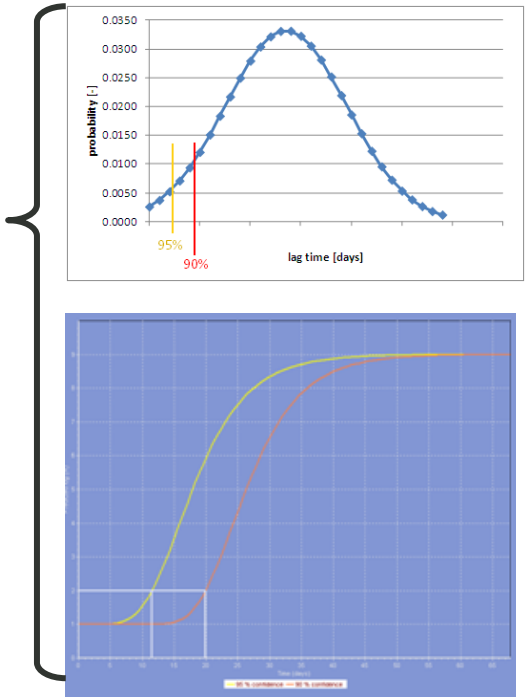
Corbion

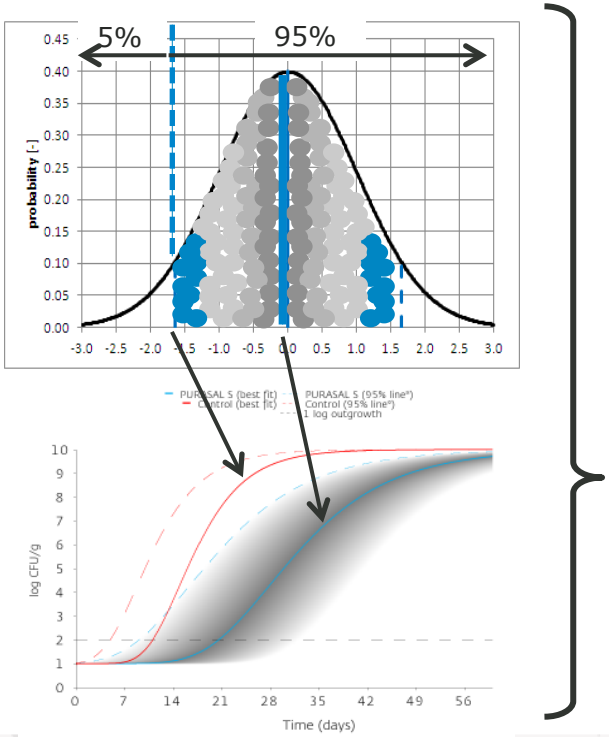
Listeria Control Model 2012

Confidence Levels

Prior to *Listeria* Control Model 2012:

- In previous versions the 90/95% confidence level was used: 90%/95% of all data is found on the right side (i.e. safe side) of the line





- In *Listeria* Control Model 2012:
  - Best fit (highest probability of outcome), i.e. 50% of data on each side of the line
  - Grey area represents 90% of data, i.e. 95% of the data lies to the right of left boundary

Corbion

Listeria Control Model 2012

Growth Prediction

Solid red: Best fit line for control (i.e. no Corbion product)

Dotted red: 95% line for control (i.e. no Corbion product)

Solid blue: Best fit with Corbion product

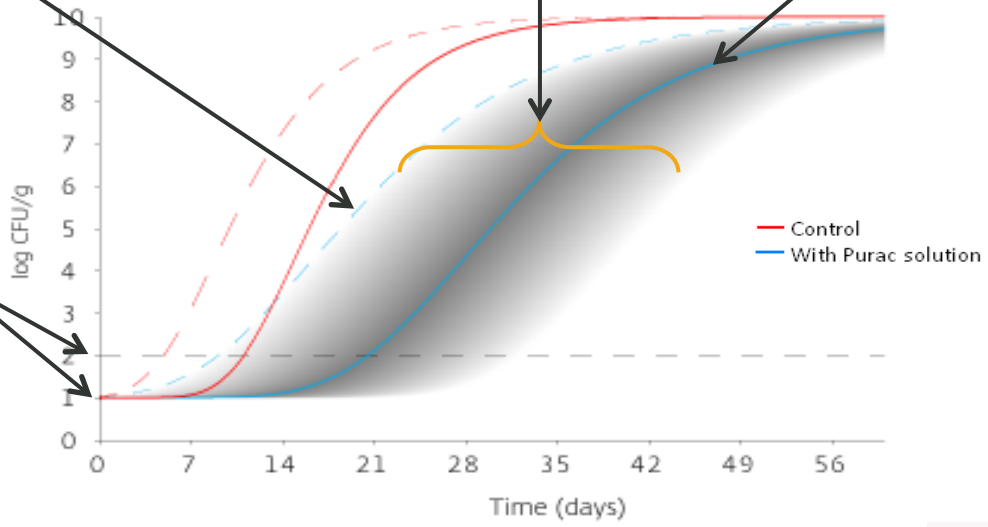
Dotted blue: 95% line with Corbion product

<u>95% Line:</u> 95% of *Listeria* growth occurs more slowly than this line, conservative estimate of growth.

<u>Grey Area:</u> where 90% of *Listeria* growth occurs

<u>Best Fit Line:</u> most likely *Listeria* growth rate

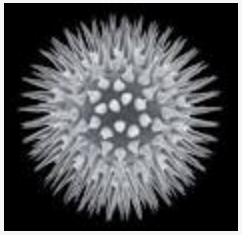
User or regulatory dictates the maximum occurrence of *Listeria* during shelf life



Corbion

Antimycotics also inhibit growth of mold

- Mold is a safety concern in grain: aflotoxin formation
- Issue in aerobically stored breads, cheese,
- Molds in meats is controlled via MAP and absence of Oxygen
- Mold Inhibitors:
  - Benzoate
  - Sorbate
  - Propionate



©

Phages= Virus

A bacteriophage is a Virus that infects and replicates within a bacteria

The term is derived from Greek word "to devour“

Composed of proteins that encapsulate a DNA or RNA genome

Phage replicate within bacteria following the injection of their genome into the cytoplasm of the host

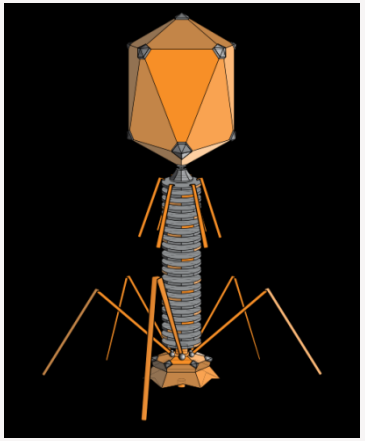
Gaining traction as both pre and post harvest intervention

Phage is very specific and must come in contact with bacterial cell to work

Will resistance develop? TBD

Spray on cattle before slaughter- e coli

Spray in package RTE – LM



©

Bacteriocins

Bacteriocins are proteinaceous toxins produced by bacteria to inhibit the growth of similar or closely related bacterial strain(s).

Nisin & Natamaycin – most popular in foods

Bind easily with fat and are rendered inactive

- hence not commonly used in meats

Expensive

Causes holes to form in bacterial cell walls

©

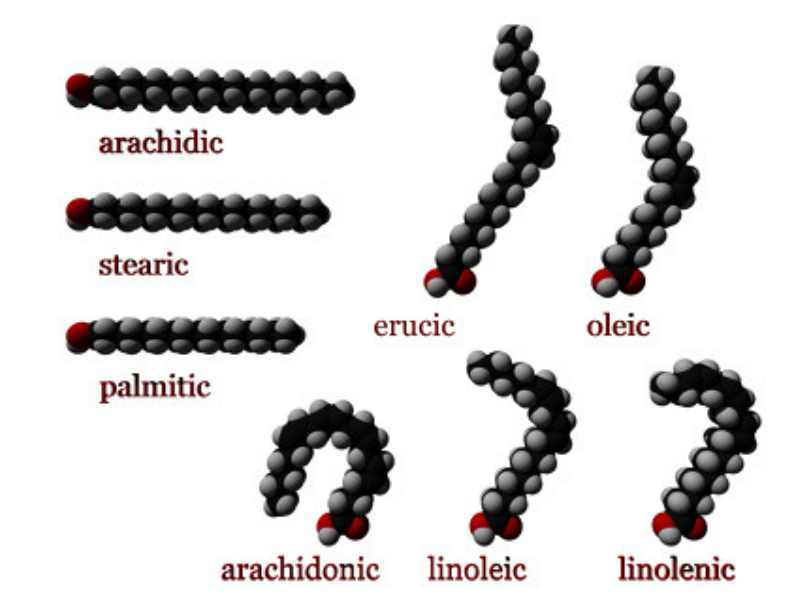
Free Fatty Acids

Lauric arginate (esterified FFA + Amino acid Arginine)

Used as topical on RTE products often applied via spray in package, sliced as well

Weak detergents, work like soap

Terminates cells ability to generate energy by damaging cell walls



©

Corbion

Summary: Ingredient Impact on Food Safety

- Most Ingredients influence safety via their effect on:
- Moisture: Aw, Ionic Strength & pH
- Antimicrobials influence safety via their effect on cellular growth
- Organic acids are the most widely used antimicrobials to inhibit growth or provide kill
- Inhibitors, while part of a HACCP Plan are not usually considered a CCP due to the fact that they do not:
  - diminish the risk of contamination
  - do not kill lm

Robin Peterson
National Accounts Manager

Purac America Inc.
7905 Quivira Road • Lenexa, Kansas 66215 • USA
T 770-664-0568 • M 847-620-9386
r.peterson@corbion.com • www.corbion.com