Microbial Control in Clean Label Products: Pathogens are the Easy Part

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Food Microbiology

• **Pathogens:**
  – *Salmonella*
  – Pathogenic *Escherichia coli*
  – *Listeria monocytogenes*
  – *Staphylococcus aureus*
  – *Clostridium perfringens, C. botulinum*

• **Spoilage Organisms:**
  – *Lactobacillus, Leuconostoc, Carnobacterium*
  – *Brocothrix, Micrococcus*
  – *Clostridium algidicarnis, C. xylanolyticum*
  – *Zygosaccharomyces, Candida, Dekkera*
  – *Penicillium, Aspergillus*
Listeria monocytogenes

- Gram-positive, rod-shaped, psychrotrophic bacterium
- Post-processing contaminant prior to or during final packaging
- If present, can grow during refrigerated storage
  - Min. growth limits
    - $a_w \ 0.92$
    - pH 4.1
• Sporeforming Bacterium
• Can grow in food/bev and produce potent neurotoxin
• Survives Pasteurization and HPP
  – HPP and 217°F would kill spores
• Minimum pH for growth 4.7
• Minimum water activity for growth 0.93
• Inhibited by natural antimicrobials
• Group I – Proteolytic and mesophilic
• Group II – Non-proteolytic and psychrotolerant
Microbial Control in Food Products

• Environment
  – SSOPs, Cleaning & Sanitization
  – GMPs, worker hygiene,

• Raw Materials & Ingredients
  – Ingredients
  – Peripheral materials (eg. packaging)

• Processing
  – “Kill step” (e.g., cooking, pasteurization, antimicrobial)
  – Cooling
  – Packaging

• Finished Product
  – “Antimicrobial agents” in formulations
  – Post-lethality treatments
Main Intrinsic Properties That Affect Microorganisms

• Moisture
  – % Moisture
  – Water activity ($a_w$)
    • Equivalent Relative Humidity
    • Vapor pressure product / vapor pressure water
  – Moisture:Protein ratio
  – % Brine Concentration (Water-Phase Salt)

• pH

• Preservatives
  – Salt
  – Lactates, diacetate, nitrite, etc.
  – Phosphates
### Water activity ($a_w$)

<table>
<thead>
<tr>
<th>Product</th>
<th>Water Activity</th>
<th>Microorganisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crackers</td>
<td>0.300</td>
<td>No Mold</td>
</tr>
<tr>
<td>Jams</td>
<td>0.65-0.7</td>
<td>Staph, Listeria</td>
</tr>
<tr>
<td>Condiments</td>
<td>0.85</td>
<td>C. botulinum, Salmonella/E. coli</td>
</tr>
<tr>
<td>Fermented Sausage</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td>Cheddar</td>
<td>0.93</td>
<td></td>
</tr>
<tr>
<td>Bologna</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td>Deli Turkey</td>
<td>0.97</td>
<td></td>
</tr>
<tr>
<td>Juice</td>
<td>0.97</td>
<td></td>
</tr>
<tr>
<td>Milk</td>
<td>0.99</td>
<td></td>
</tr>
</tbody>
</table>

Parmesan
• Most microbes grow best at neutrality
  – strain variation
  – type of acid
• Reported minimum growth pH values for some foodborne microbes
  – *Alicyclobacillus acidocaldarius* pH 2.0
  – *Penicillium roqueforti* pH 3.0
  – *Salmonella* pH 4.0
  – *Lactococcus lactis* pH 4.3
  – *Clostridium botulinum*, Group I pH 4.6
Environmental Impact for a Moderately Sensitive Formula

- **Will Spoil**
- **Might Spoil**
- **Won’t Spoil**

**Good GMPs & Plant Hygiene**
No Nitrite, Lactate/Diacetate, Dextrose (e.g., deli turkey)

**Poor GMPs & Plant Hygiene**
No Nitrite, Lactate/Diacetate, Dextrose (e.g., deli turkey)

Formula Sensitivity ———>
Options are not applicable to every product/process.

THERE ARE GOOD OPTIONS FOR CLEAN LABEL CONTROL OF MICROORGANISMS
What is the Best Food Antimicrobial?

- Greatest efficacy against microorganisms
- Lowest cost
- Most readily available (easily sourced)
- Greatest compatibility with various foods
- Multiple functionalities besides preservation

NaCl - “Salt”
Antimicrobial Processes

- Ultraviolet Light (UV)
- Heat Pasteurization
- Radiofrequency Heating
- High Hydrostatic Pressure
  - High Pressure Pasteurization (HPP)
- Pulsed Electric Field
- Cold Plasma(?)
Classes of Food Antimicrobials

• Chemical
  – **Inorganic salts**: NaCl, KCl, MgCl₂, CaCl₂, NaNO₂
  – **Organic salts**: lactate, diacetate, citrate, propioniate
  – **Organic acids**: lactic, acetic, citric, malic, tartaric
  – **Fatty acid based**: lauric arginate, octanoic acid
  – **Weak acids**: benzoic, sorbic
  – **Phenolics**: smoke fractions, synthetic phenolics
  – **Other**: hexametaphosphate, metasilicate, sulfite, EDTA
<table>
<thead>
<tr>
<th>-ic</th>
<th>-ate</th>
<th>-ite</th>
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<tbody>
<tr>
<td>citric</td>
<td>-phosphate</td>
<td>sulfite</td>
</tr>
<tr>
<td>sorbic</td>
<td>lactate</td>
<td>nitrite</td>
</tr>
<tr>
<td>acetic</td>
<td>acetate</td>
<td>metabisulfite</td>
</tr>
</tbody>
</table>
What is “Natural”?

- Acetic – Vinegar
- Benzoic – Cranberries
- Lactic – Lactic acid bacteria
- Propionic – Swiss cheese
- Sorbic – Rowanberries
Classes of Food Antimicrobials

- Plant Derived
  - Glucosinolates (Isothiocyanates) – Cruciferaceae
  - Lignans
  - Saponins (Yucca, asparagus)
  - Catechins (EGCG) – Green tea
  - Spices - essential oils & solvent extracts
### Classes of Food Antimicrobials

- **Plant Derived**
  - Spices - Essential oils & solvent extracts

<table>
<thead>
<tr>
<th>Source</th>
<th>Antimicrobial Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cinnamon</td>
<td>Cinnamic Acid, Cinnamaldehyde</td>
</tr>
<tr>
<td>Clove</td>
<td>Eugenol</td>
</tr>
<tr>
<td>Garlic</td>
<td>Allicin</td>
</tr>
<tr>
<td>Mustard</td>
<td>Allyl-isothiocyanate</td>
</tr>
<tr>
<td>Oregano</td>
<td>Carvacrol, thymol</td>
</tr>
<tr>
<td>Vanilla</td>
<td>Vanillin</td>
</tr>
<tr>
<td>Thyme</td>
<td>Thymol</td>
</tr>
<tr>
<td>Rosemary</td>
<td>α-Pinene, Camphor, Verbenone, 1, 8-Cineole</td>
</tr>
</tbody>
</table>
Classes of Food Antimicrobials

• Microbial Derived
  – Bacteriocins
    • nisin, pediocin, sakacin
  – Antimycotics
    • natamycin, chitosan
  – Fermentation products (combinations of acids, peptides & bacteriocins)
  – Live microorganisms
    • Lactic acid bacteria, *Carnobacterium maltaromaticum*
    • Phage
      – Virus that infects bacteria
      – Listeriaphage, coliphage, etc.
Aspects of Novel Antimicrobials & Sanitizers

Scientific

- Novel Clean Label Treatment
  - Efficacy
  - Natural
  - Protected & Exclusive Use
  - Certified GRAS
  - Cost & Supply
  - Legal Use
  - Consumer Acceptance
  - Product & Process Compatibility

Economic

Legal Aspects

ETNA Consulting
• Post-Pasteurization – RTE product heated in final package

• High Pressure Processing – RTE product in final package exposed to extreme hyperbaric conditions

• Topical Antimicrobials – processing aids sprayed on food surface during packaging
Clean Label
(no preservatives)

Long Shelf Life

Natural & Minimally Processed

Risk
PATHOGEN CONTROL IS WELL RESEARCHED

It is relatively “easy”
Microbiological **Safety** Risk Continuum: Producer Perspective
Most RTE Products

ORGANIC SALTS

[Chemical structures of various organic salts]
Mechanisms of Action

• Lowering of $a_w$ (e.g. from 0.985 to 0970)
  – Reduces amount of unbound water that is biologically available
Mechanisms of Action

- Weak lipophilic acids (e.g., lactic acid) pass across the cell membrane in undissociated form, dissociate within the cell and acidify the cell interior
The transcription profile in response to the combination treatment with lactate and acetate shows a shift toward fermentative production of acetoin and away from aerobic respiration and the production of lactate and acetate.

For “Clean Label” Products

VINEGAR
Behavior of *L. monocytogenes* on Natural Ham Slices as Affected by Antimicrobial Agents in Formulas During Vacuum-Packaged Storage at 4.4°C (40°F)

- □ Natural Ham with 1.05% fermentate A, 0.38% Buffered vinegar
- ■ Natural Ham No Added Inhibitors (Control)
- ● Natural Ham 2.00% Lemon Juice & Vinegar, 1.4% Fermentate A
- ○ Natural Ham with 3.36% Lactate/Diacetate Solution

Sliced ham samples (99g) were inoculated with *L. monocytogenes* (Scott A, ATCC 19111, ATCC 19115, and two plant environmental isolates) at ca. 2.5 log CFU/g. Means and standard deviation bars represent duplicate plates from duplicate samples at each time.
Inactivation of *L. monocytogenes* on hot dogs by 2ml of 5,000 ppm LAE Solution (~28 mg/kg of meat)

![Graph showing population of *L. monocytogenes* over vacuum packaged storage days with treatments: water, LAE, LAE/Smoke Flavor, and Smoke Flavor.](image-url)
Inactivation of *L. monocytogenes* on Cold-Smoked Hams 48h After Treatment with 9,090 ppm LAE Solutions

Populations of *L. monocytogenes* recovered from 3.6 kg cold-smoked hams with 1129 cm² surface areas 48 h after treatment with different volumes of water or 9,090 ppm LAE and vacuum-packaged storage at 4.4°C. Shaded bars for water (control) treatment volumes not noted with the same lowercase letters are significantly different (*P* < 0.05), and white bars for LAE treatment volumes not noted with the same uppercase letters are significantly different.
Roast beef was formulated to contain encapsulated Nisin at a level. Roast beef slices (100g per pack) were inoculated with L. monocytogenes ATCC 19115, ATCC 19111, and two environmental isolates to a target of 2.5 log CFU/g. Data points represent means of three samples.
...and is relatively misunderstood

CONTROL OF SPOILAGE ORGANISMS IS NOT WELL RESEARCHED
Food Marketing at the Speed of Business

Marketing
- Market Research
- Ideation

R&D
- Formulation
- Packaging
- Process

Food Safety & Quality
- Shelf Life Testing
- Challenge Study
- Labeling

Production
• Traditional Inhibitors
  – Sodium or potassium lactate + sodium diacetate or acetate
  – Sodium propionate

• “Natural” Inhibitors for Clean Label
  – Vinegar
  – Fruit extracts + vinegar
  – Cultured sugar (bacterial fermentates)
Leuconostoc spp.
Several Species of *Lactobacillus* Produce H$_2$S

- *Lactobacillus plantarum*, *L. fermentum*, *L. viridescens*, and various other isolates from meat products were shown to produce H$_2$S.

- Sulfurous odors in sterilized beef in vacuum-packs at 40°F for 30 days was attributed to *Lactobacillus sake* and other *Lactobacillus* spp.
0.0047 ppm is the recognition threshold, the concentration at which 50% of humans can detect the characteristic odor of hydrogen sulfide, normally described as resembling "a rotten egg"

During extended storage of meat, when glucose is depleted, homofermentative lactic acid bacteria make the metabolic switch to form end-products like acetic acid and hydrogen sulfide.

Dissolved CO₂ Slows Down the Growth of *Lactobacillus sake* on Ham

- *Lactobacillus sake* subsp. *carnosus* isolated from commercial sliced cooked ham product.
- Inoculated on cooked ham packed in various combinations of N₂ and CO₂ atmospheres and stored at refrigeration and abusive temps.

- Growth was slowed the higher the level of dissolved CO₂ in the ham.

Dissolved CO₂ Slows Down the Growth of *Lactobacillus sake* on Ham

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Growth of Lactic Acid Bacteria Including Inoculated *Lactobacillus sakei* subsp. *sakei* on Ham (Water Added) with Different Inhibitors During Modified Atmosphere-Packaged Storage at 4.4°C (40°F), Aug-Dec 2011

Sliced ham samples (99g) were inoculated with *Lactobacillus sakei* subsp. *sakei* (isolated from spoiled samples) at ca. 3.0 log$_{10}$ CFU/g. Inoculated samples were vacuum-packaged, stored at 40°F, and sampled over time by diluting with buffer, stomaching, and spiral plating on MRS agar plates. Population means and standard deviation bars represent duplicate plates from duplicate samples at each time.
Growth of Lactic Acid Bacteria Including Inoculated *Lactobacillus sakei* subsp. *sakei* on Ham (Water Added) with Different Inhibitors During Modified Atmosphere-Packaged Storage at 4.4°C (40°F), Dec 2011 - Feb 2012

Sliced ham samples (99g) were inoculated with *Lactobacillus sakei* subsp. *sakei* (isolated from spoiled samples) at ca. 3.0 log10 CFU/g. Inoculated samples were vacuum-packaged, stored at 40°F, and sampled over time by diluting with buffer, stomaching, and spiral plating on MRS agar plates. Population means and standard deviation bars represent duplicate plates from duplicate samples at each time.
• Sodium nitrite has long been used to cure meats, preserving from harmful bacteria like *Clostridium botulinum*

• Some consumers think nitrite and nitrate are bad for them, and want “natural,” “uncured,” or “no nitrites or nitrates added” products

• These products actually contain nitrate and nitrite from natural sources like celery and Swiss chard

• Acerola cherry powder
  – High in vitamin C
  – Used as natural source of antioxidants
  – Used as substitute for sodium erythorbate to accelerate the curing process
Curing “uncured” products

• Uncured products are actually cured!

• Pre-converted celery powder or other vegetables (Swiss chard) are used in the place of sodium nitrite.

• These vegetables are naturally high in nitrates and added as a natural source of nitrates/nitrites
Cured vs. Uncured

- Residual nitrite levels in finished products of uncured products are comparable to conventionally cured
  - If using pre-converted, powdered forms
  - Allows for more even distribution and color
Sporeforming and Non-Sporeforming Bacteria

- Some bacteria produce spores
- Spore = dormant stage
- Vegetative cell
Other Sporeformers that Grow at pH 5.5

- *Bacillus cereus* – can cause disease
- *Bacillus lichenformis* – can cause spoilage
- *Bacillus thermoacidurans* – can cause spoilage
- *Clostridium xylanolyticum* – can grow at refrigeration and cause spoilage
HPP of Beverages
HPP of Beverages
HPP of Beverages
• Organic, all natural, low sodium, roast beef
• Market: Foodservice
• Ingredients: cooked beef, 2% or less sea salt, sodium phosphate, natural flavor.
• Post-lethality exposed
• High-Pressure Processed (600 MPa, 4 min)
• Shelf Life: 90 days
Plate counts from Vacuum-Packaged, Sliced, Roast Beef Product Before and After High Hydrostatic Pressure Processing at 85,000 psi (600 MPa) for 180 Seconds

Microbial Population Using Various Enumeration Techniques
Validate microbial control

GET HELP AND CHALLENGE ASSUMPTIONS BEFORE LAUNCH
Two Extreme Examples of Won’t Spoil and Will Spoil

- **Will Spoil**
- **Might Spoil**
- **Won’t Spoil**

Excellent GMPs & Plant Hygiene
Nitrite, Lactate/Diacetate, Dextrose
Post Packaging Treatment

Poor GMPs & Plant Hygiene
Natural Cure, Vinegar, Sugar

Formula Sensitivity →
Sugar Impacts Spoilage Risk, Even for Non-Natural Products

Will Spoil  Might Spoil  Won't Spoil

Environmental Control

Marginal GMPs & Plant Hygiene
Nitrite, Lactate & Diacete, Dextrose

Marginal GMPs & Plant Hygiene
Nitrite, Lactate & Diacete, Sugar

Formula Sensitivity

ETNA Consulting
• CARBONATED WATER, HIGH FRUCTOSE CORN SYRUP, CITRIC ACID, NATURAL FLAVORS, SODIUM CITRATE, SODIUM BENZOATE (TO PROTECT TASTE)

• CARBONATED WATER, SUGAR, NATURAL CITRIC ACID, NATURAL FLAVORS
Food and Beverage Product Application: Many Considerations

- **Product Properties:** Liquid, Gel, Solid, Prepared meal mixture, etc.
- **Target organisms:** Yeast, mold, spoilage bacteria, pathogenic bacteria
- **Target result:** Inhibition or inactivation
• Vinegar
  – Advantages
    • Efficacy
    • History of use
    • Cost
  – Disadvantages
    • Flavor/Perception
    • Spoilage organisms not always well controlled
• **Fermentates**
  
  – **Advantages**
    
    • Combine organic acids and bacteriocins for dual mechanisms of action
    
    • Tend to be label friendly
      
      – “cultured sugar” or “cultured corn sugar”
    
    • Compatible w/various products
  
  – **Disadvantages**
    
    • Cost
    
    • Difficult to verify batch to batch consistency
    
    • Efficacy against lactics?
• Fruit Extracts & Vinegar
  – Advantages
    • Efficacy
    • Flavor
    • Label friendly
  – Disadvantages
    • Cost
    • Spoilage?
• Surface Application of Fatty Acid Based Processing Aids

• Advantages
  – Quick kill of LM (“cold pasteurization”)
  – No labeling (if below certain ppm)

• Disadvantages
  – No growth control during shelf life if there are survivors or recontamination at deli
  – Spoilage microorganisms not controlled in most cases
• Surface Application of Bacterial Culture
  – Advantages
    • Quick kill of LM (“cold pasteurization”)
    • Ongoing growth control of LM
    • Outcompetes spoilage flora
  – Disadvantages
    • Must label as “bacterial culture” or the genus species
    • CCP requirement for post-lethality treatments
    • Invalidates APC & Lactics assays used in traditional microbial shelf-life testing and environmental testing
SUMMARY

• Plenty of clean-label options for microbial control
  – Not all will work for your product
• Pathogen control is generally well-researched
• Spoilage of clean label products is not well researched, and often misunderstood
• Get help and challenge assumptions before launch
  – Validate spoilage as well as food safety