Emerging Research to Practical Approaches on Natural Antimicrobial Use

Matthew Taylor, Texas A&M University
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Food Preservation - Ancient Methods

- Fermentation, salting, burning of sulfur, vinegars
  - Acids, alcohol, plant extracts and acetate in vinegars
  - Salt
  - Sulfites, sulfur dioxide
- Dehydration/drying
- Freezing in ice
- Cooking over fire
Historical Perspectives - Food Preservative Usage

- Early 1900’s: *The Jungle*, Wiley’s Poison Squad, the Pure Food & Drug Act (1906)

- Bureau of Chemistry (within USDA): identification of food ingredients safe or unsafe for consumption in products

- Early identification of preservatives to prevent biological food deterioration
  - Na-Benzoate (first federally approved)
  - Gould amendment (1913): requirement for clear external labeling of ingredients
GRAS List and Food Additives

- First devised 1958 - Food Additives Amendment to FFDCA
- Required food manufacturers to demonstrate additives safety pre-market entry
- GRAS compounds exempted from this requirement (~200 on first GRAS list via prior-approved)
  - Data from consumer purchase and consumption without adverse response evidences
  - Other available data to industry, FDA scientists
- Current: Notification process for new additives, ingredients (GRAS Notification)
Food Antimicrobials Today

- Classification of traditional and natural antimicrobials
- Synthetic and regulatory-approved compounds
- Natural compounds (plant-derived, microbially produced)
- Globally sourced foods, ingredients (including antimicrobials) - international trade in food formulation components
- Biopreservation of foods
- Combinations of antimicrobials for food protection
- Combining antimicrobials with physical processes - *hurdle processing*

Isobologram of *L. monocytogenes* inhibition
Food Antimicrobials - Regulatory Definitions

• A type of food preservative as defined by FDA regulations

• 21 CFR §101.22(a)(5) The term chemical preservative means any chemical that, when added to food, tends to prevent or retard deterioration thereof, but does not include common salt, sugars, vinegars, spices, or oils extracted from spices, substances added to food by direct exposure thereof to wood smoke, or chemicals applied for their insecticidal or herbicidal properties.

• 21 CFR §170.3(e)(1) Food additives includes all substances not exempted by section 201(s) of the act, the intended use of which results or may reasonably be expected to result, directly or indirectly, either in their becoming a component of food or otherwise affecting the characteristics of food…

• 21 CFR §170.3(o)(2) Antimicrobial agents: Substances used to preserve food by preventing growth of microorganisms and subsequent spoilage, including fungistats, mold and rope inhibitors, and the effects listed by the National Academy of Sciences/National Research Council under “preservatives.”
Possible Concerns with Natural Antimicrobials and GRAS

- If applying for GRAS Notice, recognition, types and costs of data acquisition

- Efficacy of compound, natural preservative against targeted microbes in foods?

- Safety of antimicrobial extraction? What else comes out with antimicrobial constituents?

- *If going for preservative function, must demonstrate utility during expected shelf life*

- Interaction of extract/antimicrobial with food product
-static vs. -cidal

- Antimicrobial agents tend to be ___statics: they inhibit growth without necessarily killing cells
  - Fungistatic, bacteriostatic
  - Keeps microbial population from increasing but may not show multi-log_{10} cycle decline
  - Best at preserving against microbe population increases
- Cidal agents are killing/inactivating class of microbes
Clean Label in the FSMA Era

- Use of U.S. and non-U.S. sourced ingredients
- Foreign Supplier Verification Program
- Supply chain preventive controls installation (record-keeping needs, monitoring demands, verification of control)
- Must continue to process in sanitary fashion to produce wholesome, non-adulterated product
- Facility food safety auditing of formulation ingredients
- Others that remain to be identified!
Defining Clean Label

“Clean label is a consumer-driven movement, demanding a return to ‘real food’ and transparency through authenticity. Food products containing natural, familiar, simple ingredients that are easy to recognize, understand, and pronounce. No artificial ingredients or synthetic chemicals.”
OK: So why does all this matter? What does “clean label” mean for antimicrobial usage?

*It depends*...
Synthetic Preservatives, Sanitizers are Out!

- Weak organic acids and salts
- Sulfites
- Phosphates
- Nitrites/Nitrates (non-vegetable derived)
- DMDC
- Other examples?

Image courtesy: J. David, ConAgra Foods, Inc.
Natural Antimicrobials, Preservatives are OK, Right?

- Plant-derived antimicrobials
- Spice extracts (though not regulated as preservatives)
- Sulfurous compounds (e.g., allicin)
- Organic acids, extracts
- Microbially-derived compounds (e.g., nisin, fermentates)
- Animal-derived antimicrobials (e.g., HEWL, lactoferrin)
Positive Impacts of Clean Label

- Obvious - Respond to consumer demands/purchasing trends

- Potential to create new flavor/aroma profiles of foods without sacrifice of shelf life/safety

- Expanded product portfolios for food manufacturers

- More purchasing options for consumers of all income levels
What are Some Key Needs for Clean Label Success vis-a-vie Antimicrobial Use?

- The antimicrobial candidates integrate into/match the food
  - Replacing other acidulants for pH control
  - Humectants for $a_w$ control
  - Functional against targeted microbiota (pathogens AND spoilage alike)
- Antimicrobials
  - Purity of compound/ingredient and potency
  - Impacts of microbial growth phase on antimicrobial utility
  - Does the antimicrobial work within this food, or ineffective due to physico-chemical interactions with other food components?
Potential Negative Outcomes of Clean Label on Antimicrobial Usage

- Removal of tried-true antimicrobials, some synthetics
- Replacement with effective options that may drive consumer disliking through organoleptic impacts (e.g., buffered vinegar application to RTE meats for \( Lm \) inhibition)
- Reduced shelf-life of products?
- Needs for multiple antimicrobials/hurdles, increasing cost to consumers
- Potential for misleading/unclear labeling of a product’s composition (e.g., naturally cured meats labeled as uncured)
Potential Chemophobia Impacts

Adapted from M.P. Doyle, 2016

- Removal of benzoate, sorbate from formula of some foods
- Must regain control over fungal spoilage potential
- Benzoate exhibits anti-pathogenic activity too, so what happens to product safety?
So, What are Opportunities? How Does Research Help Us?

- Corporate R&D: Ingredient sourcing, formulation equivalency validation
- New products
- Product reformulation
- New IP, U.S. competitiveness on global markets
- Academic researchers
  - Antimicrobial activity determination, comparison, validation
  - Novel antimicrobial technology development
- Food safety validation (HACCP, HARPC) for processors
1. The research is extensive - too much to comprehensively describe today

2. The research is scattered throughout many sources: journals, books, presentations, technical documents

3. Well-devised summaries incorporating current findings are generally lacking in the academic communities
Plant-Derived Antimicrobials

• Plant extracts may be identified as GRAS for the flavoring/aroma-giving properties

• Often derived from spice plants - impacts on product organoleptic attributes

• Some are powerful antimicrobials
  • Prevent growth of pathogenic and spoilage microbiota
  • Global sources, though some stand out from others

• Used in place of traditional antimicrobials (acidulants, synthetic compounds)
Mono-Phenolics (Phenolics)

- Probably the best studied and some of the most diverse class of compounds
- Membrane destabilizing, but can also disrupt PMF and energetic processes
- Gram-positive and -negative bacteria are sensitive
- Best options:
  - Processed meats, poultry, seafood
  - Some pairings with further processed plant foods
- Complex foods

Allicin/Organo-sulfurs

- Organo-sulfur extracts from members of genus *Allium, Cruciferae*
  - Garlic, onion, shallots, chives, leeks, other species
  - Highly pungent; released from plant cells by disruption of cellular membranes
  - Can cause cell death in Gram-positive, -negative bacteria and fungi
  - Function to oxidize sulfhydryls to disulfides (enzyme catalysis loss); respiration inhibition/uncoupling from reproduction
  - Addition in product formula, or vapor phase in sealed package/process (AIT)

Image Source: wikimedia.org (Open Source)
Cinnamaldehyde Application for *E. coli* O157:H7 Control

- Non-lethal application (200 ppm) EO to pathogen for 2 or 4 hr
- Reduced expression of DNA replication genes, protein synthesizing genes, LPS construction protein genes (2 hr)
- Increased stress response gene expression at 2 hr; lost at 4 hr
Addition of Pepper, Parsley, or Dill Extracts for Cheese Preservation

- Kareish cheese (soft cheese, low fat, Egypt)
- Extracts of plants added to cheese post-pasteurization
- Determination of anti-\textit{S. aureus} activity of extracts in cheese during post-treatment storage
- (Foodborne Pathog. Dis. 2010. 7:411)
Hurdle Processing with Natural Antimicrobials

- Current, recent research examining effect of combined antimicrobials or processes on pathogen/spoilage microbe control in foods

- Combinations and hurdle processing may reduce overall antimicrobial utilization without safety/quality detriment

- Pairing antimicrobials with physical processing (thermal, non-thermal)
Pairing Essential Oils with Thermal Processing for Food Safety

- Addition of clove or cinnamon EO to apple cider
- Impacts on *E. coli* O157:H7 death kinetic (D-value)
- *J. Food Prot.* 2007. 70:2089

- EO infusion into ground beef + cooking for *E. coli* O157 destruction
- *Front. Microbiol.* 2016. 7(15):1

**TABLE 5. D-values of *E. coli* O157:H7 in apple cider with and without cinnamon and clove essential oils**

<table>
<thead>
<tr>
<th>Temp (°C)</th>
<th>Cider alone</th>
<th>Cider + 0.01% cinnamon oil</th>
<th>Cider + 0.01% clove oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>46</td>
<td>120 A</td>
<td>61.1 B</td>
<td>61.3 B</td>
</tr>
<tr>
<td>48</td>
<td>64.4 A</td>
<td>58.9 A</td>
<td>35.7 B</td>
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<tr>
<td>50</td>
<td>21.8 A</td>
<td>11.6 B</td>
<td>7.38 B</td>
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<tr>
<td>52</td>
<td>15.5 A</td>
<td>9.12 A</td>
<td>3.09 B</td>
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<tr>
<td>54</td>
<td>3.91 A</td>
<td>4.56 A</td>
<td>2.86 A</td>
</tr>
</tbody>
</table>

\(^a\) Within the same row, values not sharing the same letter are significantly different (\(P \leq 0.05\)).
Combining Antimicrobials and Opportunity for Synergism

- Pairs or even three-compound applications have been reported to demonstrate synergistic inhibition of microbes
- Applications for processed meats, seafoods, formulated products
- J. Food Sci. 2010. 75(9):M557
Inhibition of Spoilage Microbiota via Combined Natural Antimicrobials

Table 3 – Combination effects of antimicrobials against selected strains *in vitro*

<table>
<thead>
<tr>
<th>Component A</th>
<th>Component B</th>
<th>Thy Cln</th>
<th>Thy Clt</th>
<th>Thy Ros</th>
<th>Thy AIT</th>
<th>Asc Cln</th>
<th>Asc AIT</th>
<th>AIT Asc</th>
<th>AIT Clt</th>
<th>GSE Clt</th>
<th>GSE Cln</th>
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</thead>
<tbody>
<tr>
<td>Leuc. carnosum</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<td>++</td>
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<tr>
<td>Leuc. mesenteroides</td>
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<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
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</tr>
<tr>
<td>C. maltaromaticum</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>C. divergens</td>
<td>+</td>
<td>++</td>
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<td>++</td>
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<tr>
<td>Lact. algidus</td>
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<td>++</td>
<td>-</td>
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<td>-</td>
<td>+</td>
<td>++</td>
<td>+</td>
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<tr>
<td>B. thermosphacta</td>
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<tr>
<td>S. proteamaculans</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
</tbody>
</table>

*Concentration range: thymol (Thy): 0 to 600 µg/mL, ascorbic acid (Asc): 0 to 40000 µg/mL, allyl isothiocyanate (AIT): 0 to 8000 µg/mL, grape fruit seed extract (GSE): 0 to 2000 µg/mL.*

*Concentration range: cinnamaldehyde (Cltn): 0 to 250 µg/mL, citric acid (Cln): 0 to 3000 µg/mL, rosemary extract (Ros): 0 to 150 µg/mL, allyl isothiocyanate (AIT): 0 to 2000 µg/mL, ascorbic acid (Asc): 0 to 2500 µg/mL.*

+++: synergistic effect (FIC < 0.5), ++: some synergistic effect (0.5 < FIC < 0.75), +: additive effect (0.75 < FIC < 1.0), -: no correlation of effects (FIC > 1.0).

J. Food Sci. 2010. 75(2): M98

Combined natural antimicrobials against pork meat spoilage microbes.
Delivering Antimicrobials via Encapsulation

- Research-wise, a hot trend, with some industry applications (e.g., encapsulated citric acid)
- Particularly useful for hydrophobic plant-derived antimicrobials
- Can use natural polymers/encapsulate systems
  - Pectins and derivatives
  - Starches (modified, non-modified)
  - Whey protein extract, soy protein extract/isolate
  - Lipids/emulsifiers (some naturally occurring)
Biopreservation for Food Safety Protection

• Ananou et al. 2007: The extension of shelf life and enhanced safety of foods by use of natural or controlled microbiota and/or antimicrobial compounds

• Three key forms/applications of biopreservatives:

  • Fermentative non-pathogenic microbes, principally the lactic acid bacteria (LAB)
  
  • Fermentates from non-pathogenic fermentative microbes, purified and added to other foods (e.g., acids, bacteriocins, etc.)

  • Bacteriophages
Biopreservative Products and Regulatory Approvals

- Antimicrobial interventions incorporating viable LAB (single species or multi-organism product) - USDA-FSIS
  - *Carnobacterium maltaromaticum* (Approved for RTE meats as antilisterial agent in U.S., Canada)
  - *Lactobacillus, Pediococcus* spp. (Approved for fresh and processed meat safety by FDA/USDA)

- FDA has provided GRAS affirmation for many LAB products used in fermentation processing, but very few approvals in biopreservatives
Antimicrobial Metabolite Products

- Produced via industrial fermentations, captured and purified/semi-purified, stabilized and packaged
- Approved for application across various food product types
- Must be produced by known non-pathogenic microbes
- Comprised by some combination of: acids, antimicrobial peptides, peroxides, misc antimicrobials
- Examples:
  - Nisin preparation (GRAS 1988)
  - Mixed fermentates
  - Natamycin (antifungal)
  - Poly-L-lysine
Bacteriophages

- Predatorial, parasitic viruses of limited range of bacteria
- High host-specificity
- Theoretical “one hit, one kill”
- Ongoing co-evolution of host and parasite against one another
- First GRAS approved for *L. monocytogenes* control on RTE foods, 2006
- Multiple approved for food and food animal applications for reducing pathogens
FSIS Directive 7120.1, Rev. 40

<table>
<thead>
<tr>
<th>Substance</th>
<th>Product</th>
<th>Amount</th>
<th>Reference</th>
<th>Label Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre- and/or Post-Harvest Utilization Related to STEC Decontamination</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteriophage preparation (<strong>E. coli O157:H7 targeted</strong>)</td>
<td>On the hides of live animals (cattle) in the holding pens prior to slaughter and hide removal</td>
<td>Applied as a spray, mist, rinse or wash to the hides of live animals (cattle) within lairage, restraining areas, stunning areas, and other stations immediately prior to hide removal</td>
<td>Acceptability determination</td>
<td>None under the accepted conditions of use (1)</td>
</tr>
<tr>
<td>Bacteriophage preparation (<strong>E. coli O157:H7 targeted</strong>)</td>
<td>Red meat parts and trim prior to grinding</td>
<td>Applied as a mixture diluted with water at a ratio of 1:10</td>
<td>FCN No. 1018</td>
<td>None under the accepted conditions of use (1)</td>
</tr>
</tbody>
</table>
Summary

• Clean label opens a lot of doors, but closes some too

• Antimicrobials are particularly impacted as preservatives, but opportunities for new formulation are identified

• Of course, one cannot sacrifice safety/wholesomeness just for clean(er) label

• In addition to some traditional antimicrobials, there are options of natural antimicrobials, as well as some biopreservation agents that may be useful in clean label development
Conagra Antimicrobial Toolbox

Classical Chemical Preservatives

- Organic Acids & Salts
  - Sorbate
  - Benzoate
  - Propionate
  - Lactate
  - Diacetate
  - Citrate
  - Nitrate/Nitrite

- Bacteriocins
  - Nisin
  - Natamycin (Natural Mold Inhibitor)

- Live Cultures
  - Lactic Starter Culture
  - Yeast Spray
  - Bacteriophages
  - (Processing Aid)

- Fermentates
  - Cultured Cane Sugar
  - Cultured Sugar with Vinegar
  - Cultured Dairy
  - Cultured Wheat Starch

- Natural Ingredients
  - Plant or Plant Extracts
  - Celery Juice Powder Extract
  - Cherry Powder
  - Rosemary
  - Vinegar
  - Essential Oils
  - Phyto-Phenols
  - Bioflavonoids

“NATURAL”

“CLEAN LABEL”

• “ate” List
• These are perfectly safe!!

— CONAGRA RQI TECH REVIEW 21 APRIL 2016
Thanks for listening!

Any questions?