Opportunities and Limitations of "Natural" Antimicrobials

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CLEAN LABEL CONFERENCE

Overview

- Food antimicrobials: definition and use
- Clean label antimicrobial ingredients
- Factors affecting efficacy of antimicrobials
- Verification of efficacy
- Summary: considerations for formulating for safety

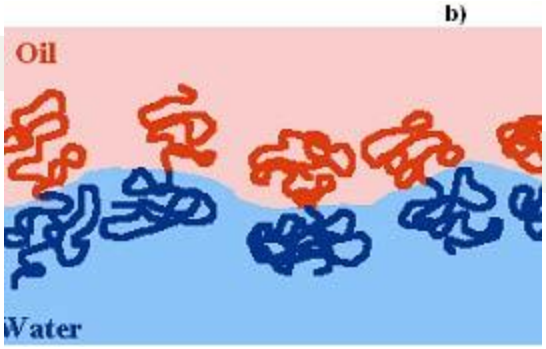
Defining food antimicrobials

- Ingredient/organism which inhibits growth or inactivate pathogenic or spoilage microorganisms
- Primary: food safety
  - Supplement current good manufacturing practices
  - Control microorganisms throughout the food chain
  - Reduce growth of pathogens in processed foods or reduce contamination levels of raw foods
  - Reduce foodborne illness
- Secondary: shelf-life extension

Effectiveness as an antimicrobial

- Concentration of active compounds
- Solubility of antimicrobial
- Dissociation constant
- Food composition
  - Fat, moisture, hydrophobic proteins, free iron, pH
  - Salt content/water activity/availability
- Synergistic/additive effects between antimicrobials
- Processing, cooling and storage temperature/time

Key Characteristics



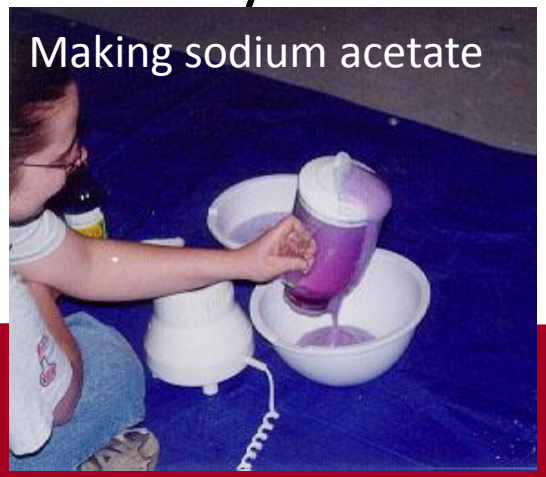
- Amphiphilic
  - Lipophilic: Attach and pass through cell membrane
  - Partially soluble in aqueous phase
- Antagonistic interaction with food components
  - Partition into fat phase; reduces activity
  - Bind to hydrophobic proteins
- Often associated with flavor compounds
- More effective at lower pH values

Conventional antimicrobials

- NaCl
  - Reduces available water (a_w)
- Organic acids and their salts
  - lactate, acetate, diacetate
- Antimycotics (acid and salt forms)
  - sorbate, benzoate, propionate
- Nitrite
- Phosphates; some antioxidants

Defining “natural” antimicrobials

- Naturally occurring compounds
- “directly extracted using simple methods, chemical reactions or naturally occurring biological process”
  - No petrochemicals, genetic engineering
- “any substance derived from a living organism”
  - Biopreservatives
- “no processing beyond what you could do in your own kitchen”



Antimicrobials from “natural” source

- Microbial
  - Fermentation byproducts
    - Organic acids and other primary metabolites
    - Bacteriocins (nisin)
  - Competitive cultures
  - Bacteriophages
  - Natamycin (pimaricin)
- Minerals and gases
  - NaCl
  - 100% CO_2, CO
- Plants
  - Spices, extracts, essential oils, oleoresins
  - Natural wood smoke components
  - “natural nitrate/nitrite”
  - Fatty acids
- Animal
  - Lysozyme
  - Chitosan
  - Lactoferrin
  - Milk lactoperoxidase

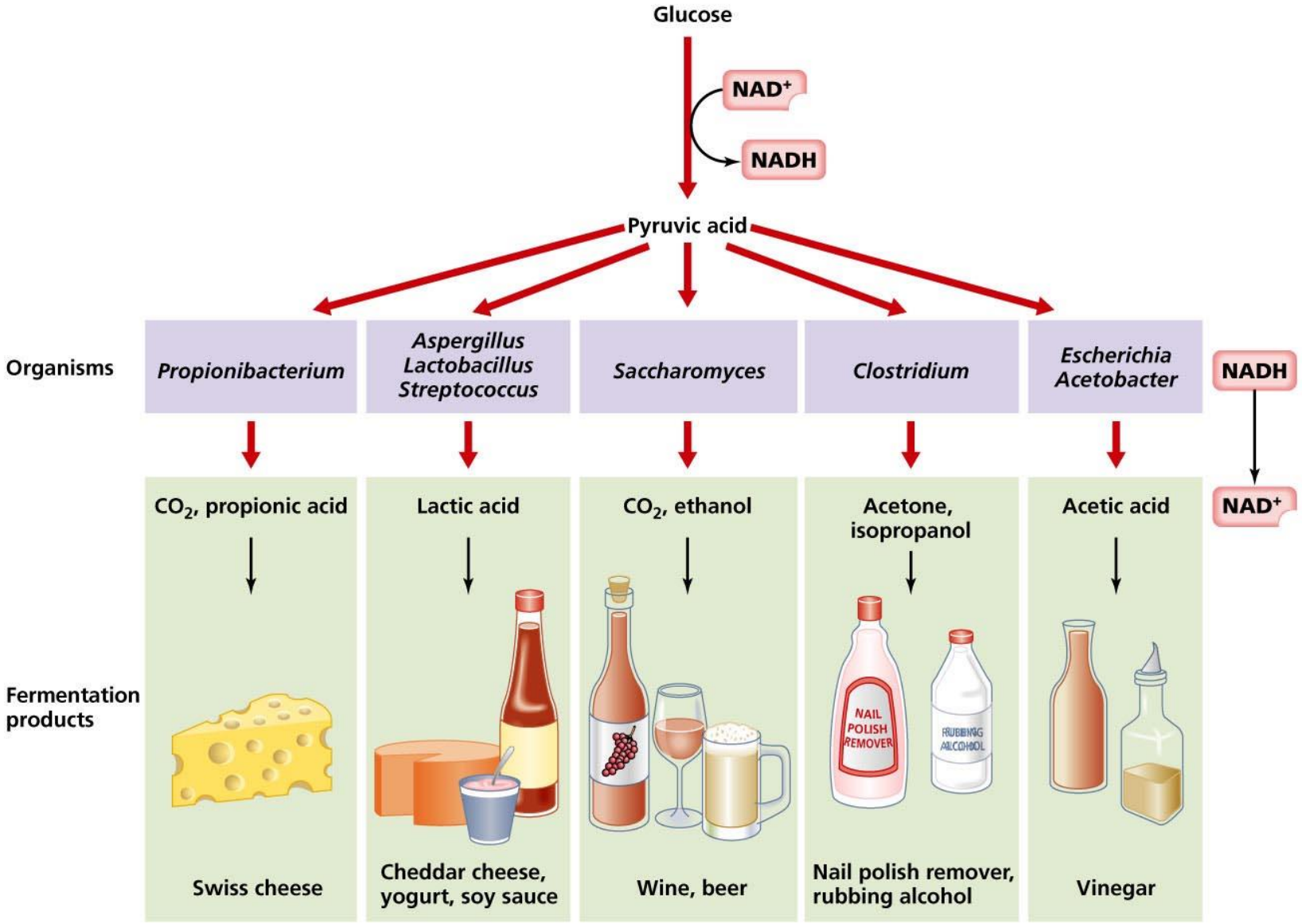
Clean Label Alternatives

E number	Common Name	Clean label version
E260	Acetic acid	Vinegar
E280	Propionic acid	Culture sugar/ dairy solids
E270	Lactic acid	Culture sugar/ dairy solids
E234	Nisin (bacteriocins)	Culture sugar/ dairy solids
E300	Ascorbic acid	Cherry powder
E392	Extracts of rosemary	Rosemary
E1105	Lysozyme	Egg white
E250	Sodium nitrite	Cultured vegetable juice
E251	Sodium nitrate	Celery, spinach

Per Regulation (EC) No 133/2008, considered food additives

Fermentates

- Commercially available, proprietary ingredients
- Culture sugar or milk; spray dry
- Blends of organic acids
  - Lactic acid, propionic acid, acetic acid
- May or may not contain bacteriocin activity
- Byproducts depend on:
  - Starter culture(s) used
    - *Propionibacterium, Lactococcus, Pediococcus,* etc.
  - Substrate
  - Controlled fermentation
    - Temperature, oxygen, nutrient availability

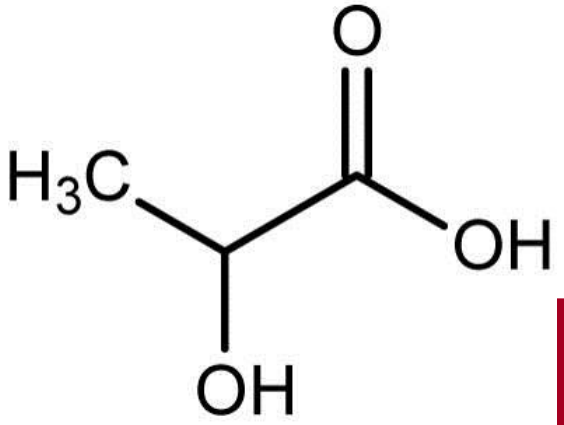


Organic Acids

- Mechanism:
  - Undissociated form lowers internal pH
    - Protein denaturation
    - Disrupt proton motive force; inhibit membrane transport; starve cells
  - Chelate metal ions
  - Can cause sub-lethal injury
    - Enhance efficacy of other antimicrobials

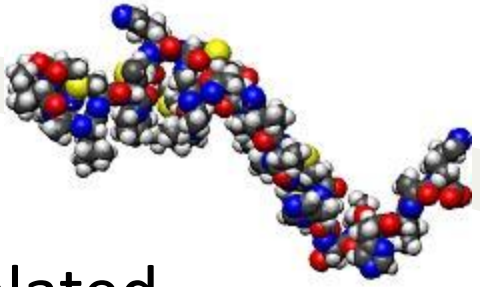
Organic Acids and Salts

- Optimizing efficacy
  - Lower pH values (e.g. <5.5; near pK_a)
  - Lower temperatures (4 vs. 7 or 10°C)
    - Exception: pH ≤4.6; then combined stress with higher temperatures increases inactivation rate
  - Combined with other antimicrobials

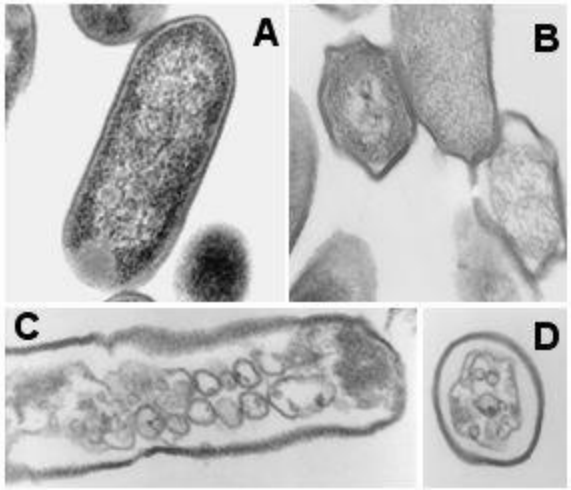


Lactic acid

Bacteriocins



- Polypeptides that inhibit other closely related species
- Byproduct of lactic acid bacteria fermentation
  - Nisin, pediocin, reuterin
  - Active against Gram positive bacteria
- Mechanism: bind to receptor; pore formation; leakage of molecules, cell death



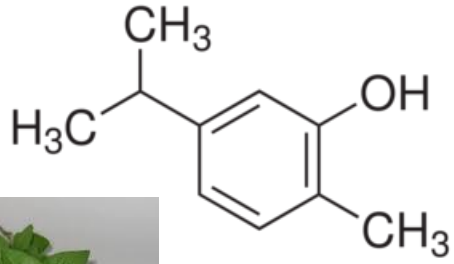
L. monocytogenes cell damage by bacteriocin
Mendoza et al., 1999

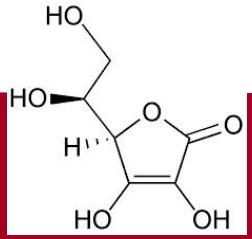
Bacteriocins

- Advantages: bactericidal
- Disadvantages:
  - Proteolytic enzymes in raw foods may inactivate bacteriocin
  - Resistance development by microbes
  - Less effective in high-fat foods
  - May inhibit beneficial competitive microflora
- Works best:
  - Low fat; pH <6
  - In combination with other antimicrobial

Plant extracts/spices/glycerides

- Native compounds, protect plant
- Extracted with water or ethanol; concentrated
- Common uses in foods
  - Flavors: cinnamon, thyme, mustard, cloves, oregano
  - Antioxidants: dried plum, rosemary, tocopherol (Vit E), ascorbate (Vit C)
  - Emulsifiers: monoglycerides, sucrose monolaurate
- Mechanism: Disrupt cell membrane
- Efficacy as antimicrobial: 0.5% - 5%



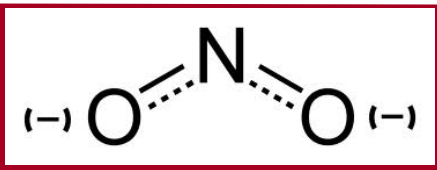


Plant extracts/spices/glycerides

- Disadvantages:
  - Variability due to variety/extraction/agricultural practices
  - Partition into fat phase
  - Strong odor; flavor; color
  - Unknown toxicological effect at higher concentrations
  - Activity decrease after heating (carrot, allicin)

Nitrite: derived from cultured celery

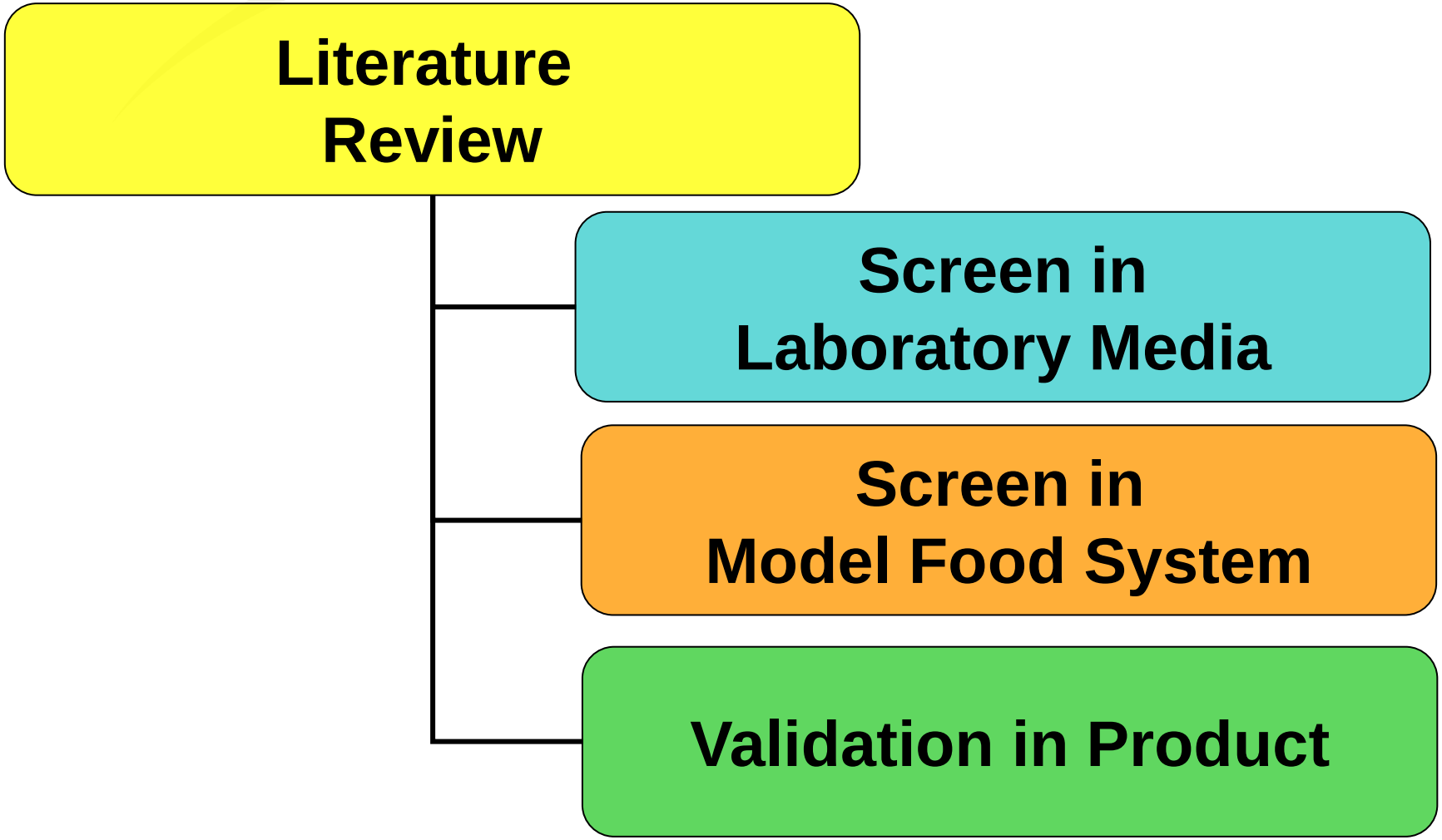
- Reduce NO_3 to NO_2 with nitrate reducing starter
- Activity against *Clostridium sp., L. monocytogenes*
- Mechanism:
  - Inactivate iron-sulfur enzymes by binding of nitric oxide
  - Inhibit bacterial respiration
- Greater activity in presence of ascorbate, fermentates
- Applications: cured meat; color (reacts with myoglobin to give cured meat color); flavor; antimicrobial
- Comply with regulatory limits for synthetic nitrite



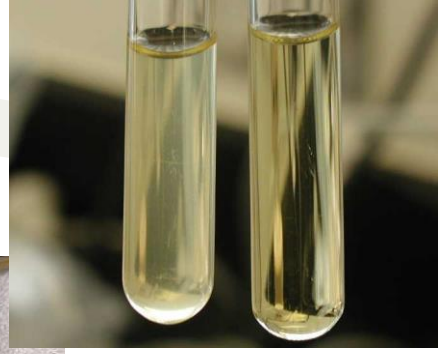
Evaluating Efficacy: Getting Started

- Understand mechanism of action
- Understand factors affecting efficacy
  - Solubility/polarity
  - Dissociation constant – product pH
  - Additive and synergistic effects
  - Temperature
  - Stability to heat; enzymes; water
  - Potential for resistant populations
  - Potential to inhibit growth of competitive spoilage bacteria that reduce pH

Approaches to Determine Efficacy



Screening in Media

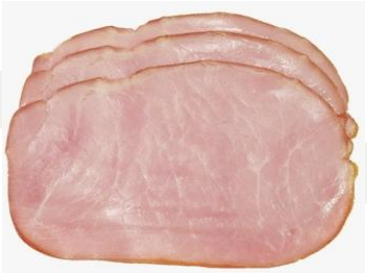


- Assays
  - Turbimetric
  - Agar diffusion
  - Kill
- Effect of diluent and preparation
  - Ethanol vs. alternate diluents for non-water soluble
  - Heat
- Effect of pH of media
  - Adjust to that relevant to foods
  - Buffering
  - pK_a of antimicrobial

Screening in Model Food Systems

- Consider protein, fat, carbohydrates
  - Binding of antimicrobial
  - Nutrient source
  - Interactive effects w/other antimicrobials
- Milk
  - Fat levels
- Meat suspensions / slurries
  - Adjust pH; brine; nitrite; phosphate
- No or low levels competitive microflora

Validation in Product



- Complete under guidance of expert food microbiologist
- Study design, controls, replication
- Replicate typical route of contamination
- Replicate processing conditions
  - Availability of equipment
- Storage conditions
- Product/Production variation
- Microbial and proximate analysis
- Shelf-life and sensory attributes
- Interpretation and measure of “success”

Protective cultures

- Competitive exclusion
- Production of bacteriocins
- Can be used effectively in conjunction with other preservatives
- Effective when product may be temperature abused

Challenges: Optimizing Efficacy

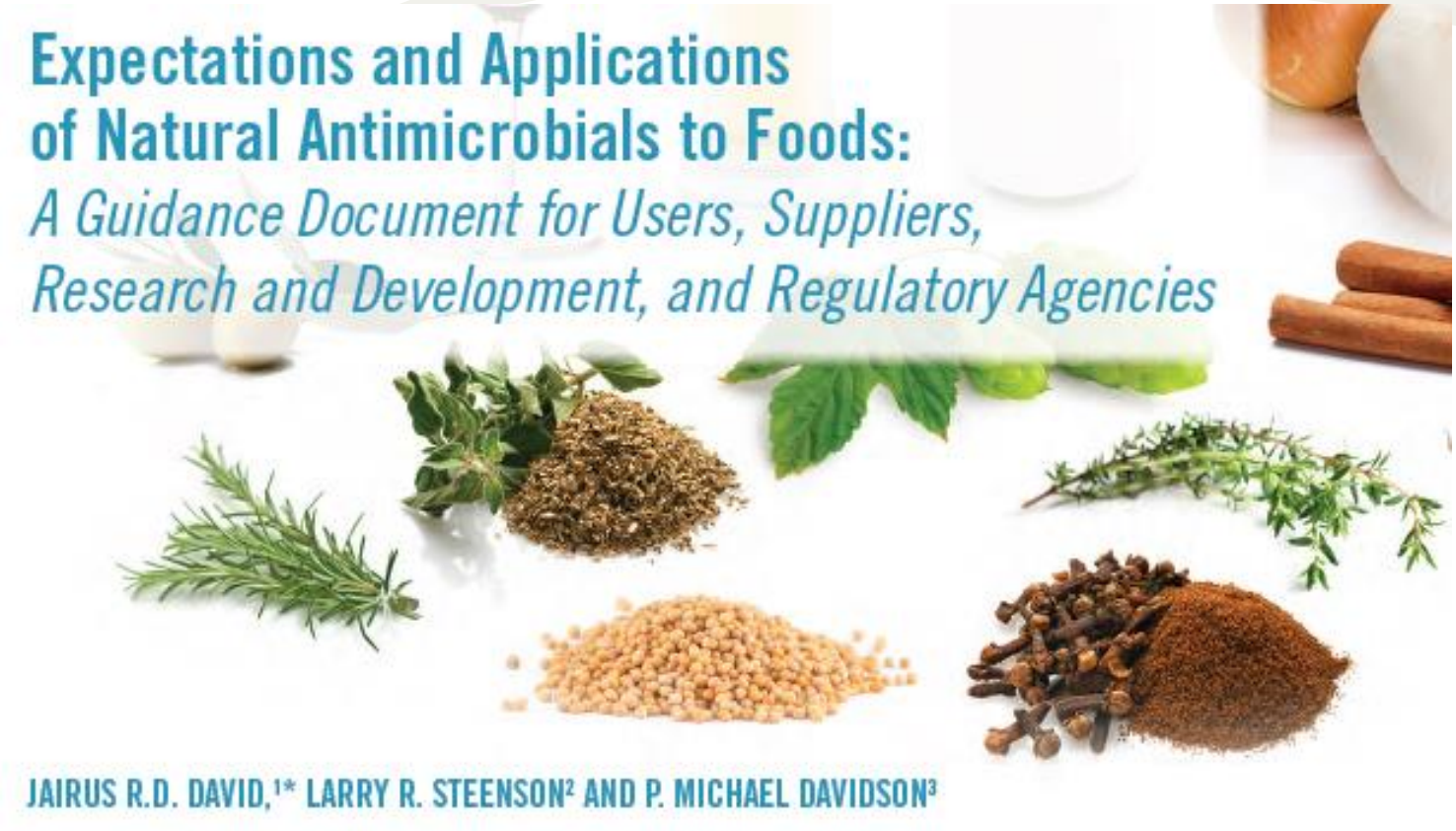
- Use in conjunction with standard food safety practices
- Recognize effect of processing, distribution, and storage temperature
- Determine target organism of concern
- Consider binding of antimicrobial food components
- Modify formulations to inhibit growth
  - pH, water activity
  - Other antimicrobial ingredients

Limitations

- Concentrations required for antimicrobial activity may negatively affect sensory attributes
- Undefined activity between manufacturers; lots
- Affected by processing, food components, temperature control, intrinsic factors (aw, pH)
- Single antimicrobial will not control all microbes
- Other quality issues/shelf-life
- Must be validated in specific foods
- Business decision; cost-benefit

Opportunities

- Clean label antimicrobials can be applied to wide variety of foods
- Ingredients familiar to consumer can enhance the safety of foods
  - Adjunct to good manufacturing practices
  - Confidence by consumers
- Optimization of ingredients can reduce usage levels, improve sensory attributes and be cost-effective



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Thank you for your attention.

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