Antimicrobial Ingredients

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Antimicrobial Ingredients

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Clean Label Product Innovation Short Course
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Objectives

▪ To review the factors that affect microbial growth in foods
▪ To present antimicrobial ingredients that may be considered “clean label”
▪ To present and discuss some of the challenges associated with “clean label” antimicrobials
Agenda

- Microbial growth in foods
- Food parameters that impact microbial growth in foods
- Strategies for microbial control in foods
- Antimicrobials in foods
- “Clean label” antimicrobials
- Current challenges of “clean label” antimicrobials
Microbial Growth in Foods

▪ Spoilage Microorganisms
  • Reduction of keeping quality and shelf-life → economic losses; food waste; loss of consumer confidence and trust

▪ Pathogenic Microorganisms
  • Consumer discomfort, illness or death → human pain, loss and grief; lost productivity; increased health care costs; decreased consumer confidence and trust in the food industry and the food supply

▪ Except post-package sterilized foods, all foods are subject to food spoilage or loss of microbiological safety if ideal microbial growth conditions are present.
General Pattern of Microbial Spoilage

Food Parameters that Influence Microbial Growth

<table>
<thead>
<tr>
<th>Intrinsic Parameters</th>
<th>Extrinsic Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>Inactivation temperature</td>
</tr>
<tr>
<td>Available Moisture</td>
<td>Storage temperature</td>
</tr>
<tr>
<td>Nutrients</td>
<td>Storage atmosphere</td>
</tr>
<tr>
<td>Antimicrobial constituents</td>
<td></td>
</tr>
<tr>
<td>Oxidation-Reduction (O/R) Potential (Eh)</td>
<td></td>
</tr>
<tr>
<td>Biological structures</td>
<td></td>
</tr>
</tbody>
</table>
pH

- $\text{pH} = -\log [\text{H}^+]$
- Microbes try to maintain pH within narrow range $\rightarrow$ “homeostasis”
- Intracellular pH typically near neutral
- Microbial classification by optimal pH range:
  - Acidophiles $\rightarrow$ pH 0.5–5
  - Neutralophiles $\rightarrow$ pH 5–9
  - Alkaliphiles $\rightarrow$ pH 9–13
- Major deviations from optimal pH disrupt pH homeostatic mechanisms
- Cytoplasmic membrane relatively impermeable to $\text{H}^+$ and $-\text{OH}$ ions
Available Moisture

- Described by water activity ($a_w$)
  \[ a_w = \frac{p}{p_o} \]  
  (vapor pressure of food)  
  (vapor pressure of pure water)

- Related to equilibrium relative humidity (ERH) above the food
  \[ ERH = a_w \times 100 \]

- Describes degree to which water is bound in food

- Measures availability of water to support microbial growth and participate in chemical/biochemical reactions

- $a_w$ not the same as moisture (or water) content, or percent moisture
Effect of $a_w$ on chemical and microbial stability of foods

![Graph showing the effects of water activity on various processes](image-url)
## Minimum $a_w$ requirements for microbial growth

<table>
<thead>
<tr>
<th>Type of Microorganism</th>
<th>Water Activity ($a_w$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>0.85–1.00</td>
</tr>
<tr>
<td>Most yeasts</td>
<td>0.87–0.91</td>
</tr>
<tr>
<td>Molds</td>
<td>0.80–0.87</td>
</tr>
<tr>
<td>Certain yeast and molds</td>
<td>0.71–0.86</td>
</tr>
<tr>
<td>Certain yeasts</td>
<td>0.60–0.70</td>
</tr>
<tr>
<td>None</td>
<td>&lt;0.60</td>
</tr>
</tbody>
</table>
### $a_w$ of Selected Foods

<table>
<thead>
<tr>
<th>Food</th>
<th>$a_w$</th>
<th>Food</th>
<th>$a_w$</th>
<th>Food</th>
<th>$a_w$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh meat and fish</td>
<td>0.99–1.00</td>
<td>Baked cake</td>
<td>0.90–0.94</td>
<td>Fruit cake</td>
<td>0.73–0.83</td>
</tr>
<tr>
<td>Fresh fruits &amp; vegetables</td>
<td>0.97–1.00</td>
<td>Jams and jellies</td>
<td>0.75–0.94</td>
<td>Hard salami</td>
<td>0.82</td>
</tr>
<tr>
<td>Natural cheeses</td>
<td>0.95–1.00</td>
<td>Bean paste</td>
<td>0.93</td>
<td>Beef jerky</td>
<td>0.78–0.82</td>
</tr>
<tr>
<td>Fluid milk</td>
<td>0.99</td>
<td>Caviar</td>
<td>0.92</td>
<td>Soy sauce</td>
<td>0.80</td>
</tr>
<tr>
<td>Pudding</td>
<td>0.97–0.99</td>
<td>Summer sausage</td>
<td>0.88</td>
<td>Dried fruit</td>
<td>0.55–0.80</td>
</tr>
<tr>
<td>Bacon</td>
<td>0.97</td>
<td>Uncooked rice</td>
<td>0.80–0.87</td>
<td>Honey</td>
<td>0.75</td>
</tr>
<tr>
<td>Cooked Ham</td>
<td>0.97</td>
<td>Flour</td>
<td>0.67–0.87</td>
<td>Peanut butter, 15% moist</td>
<td>0.70</td>
</tr>
<tr>
<td>Eggs</td>
<td>0.97</td>
<td>Aged cheddar cheese</td>
<td>0.85</td>
<td>Dried whole egg</td>
<td>0.40</td>
</tr>
<tr>
<td>Pork sausage</td>
<td>0.97</td>
<td>Maple syrup</td>
<td>0.85</td>
<td>Cookies</td>
<td>0.30</td>
</tr>
<tr>
<td>Frankfurter</td>
<td>0.96–0.97</td>
<td>Fruit juice concentrates</td>
<td>0.79–0.84</td>
<td>Instant coffee</td>
<td>0.20</td>
</tr>
<tr>
<td>Liverwurst</td>
<td>0.96–0.97</td>
<td>Cake icing</td>
<td>0.75–0.84</td>
<td>Dried whole milk</td>
<td>0.20</td>
</tr>
<tr>
<td>Bread, white</td>
<td>0.94–0.97</td>
<td>Fudge sauce</td>
<td>0.83</td>
<td>RTE breakfast cereal</td>
<td>0.10–0.20</td>
</tr>
<tr>
<td>Cheese spread</td>
<td>0.95</td>
<td>Sweetened cond. milk</td>
<td>0.83</td>
<td>Crackers</td>
<td>0.10</td>
</tr>
</tbody>
</table>
Nutrients

Nutrient requirements of microorganisms

1. Water
2. Energy → primarily sugars, alcohols, amino acids
3. Nitrogen → amino acids
4. Vitamins and growth factors
5. Minerals

Requirements, least to highest:

• Molds → yeasts → gram-negative bacteria → gram-positive bacteria
Oxidation-Reduction (O/R) Potential (Eh)

- Ratio of oxidizing vs. reducing power of a substance
- Eh of a substance measured in mV (pH 7.0; 30°C)
- \( \downarrow \text{O}_2 \rightarrow \downarrow \text{Eh} \)
- Can be extrinsic parameter also (i.e., packaging atmosphere)
- Classification of microorganisms based on Eh requirements:

<table>
<thead>
<tr>
<th></th>
<th>Eh range (mV)</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobes</td>
<td>[+500] – [+300]</td>
<td><em>Aeromonas hydrophila</em></td>
</tr>
<tr>
<td>Facultative anaerobes</td>
<td>[+300] – [-100]</td>
<td><em>Escherichia coli</em></td>
</tr>
<tr>
<td>Anaerobes</td>
<td>[+100] – [-250]</td>
<td><em>Clostridium botulinum</em></td>
</tr>
<tr>
<td>Microaerophiles</td>
<td></td>
<td><em>Campylobacter jejuni</em></td>
</tr>
</tbody>
</table>
Biological Structures

- Natural protective coverings of foods
  - e.g., egg shells, nut shells, fruit peels, seed coats, animal hides/skin
- In intact form, provide physical barrier to entry of microorganisms
### Temperature

Classification of microbes based on temperature requirements:

<table>
<thead>
<tr>
<th></th>
<th>Growth range (°C)</th>
<th>Growth optima (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psychrophiles</td>
<td>[-5]–20</td>
<td>10–15</td>
</tr>
<tr>
<td>Psychrotrophs</td>
<td>&gt;0</td>
<td>n/a</td>
</tr>
<tr>
<td>Mesophiles</td>
<td>10–45</td>
<td>35–40</td>
</tr>
<tr>
<td>Thermophiles</td>
<td>45–70</td>
<td>55–60</td>
</tr>
</tbody>
</table>
Effect of temperature on growth rate of microorganisms

- **Growth rate**
- **Temperature**

<table>
<thead>
<tr>
<th>Minimum</th>
<th>Optimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membranes gel; transport processes too slow for growth</td>
<td>Enzymatic reactions at maximal possible rate</td>
<td>Proteins denature; cytoplasmic membrane collapses; thermal lysis</td>
</tr>
</tbody>
</table>
Temperature

- Ways to control microorganisms:
  - High temperature: cooking, canning, etc.
  - Low temperature: refrigeration, freezing, etc.
  - Avoid temperature “danger zone” (40–140°F)

Graphic source:
Antimicrobial Constituents

- May be:
  - Naturally-present
  - Produced by competitive microflora
  - Deliberately added as food additives
## Microbial growth control strategies

<table>
<thead>
<tr>
<th>Control barrier</th>
<th>Objective</th>
<th>Example strategies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inactivation temperature</td>
<td></td>
<td>Cooking; canning</td>
</tr>
<tr>
<td>Storage temperature</td>
<td></td>
<td>Refrigeration; freezing</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td>Fermentation; acidification</td>
</tr>
<tr>
<td>O/R Potential (Eh)</td>
<td></td>
<td>Vacuum or modified atmosphere</td>
</tr>
<tr>
<td>Water activity (a&lt;sub&gt;w&lt;/sub&gt;)</td>
<td></td>
<td>Dehydration; addition of salt or sugar</td>
</tr>
<tr>
<td><strong>Antimicrobial ingredients</strong></td>
<td></td>
<td>Lactate; sorbate; diacetate; vinegar; fermentates; etc.</td>
</tr>
<tr>
<td>Competitive microflora</td>
<td></td>
<td>Starter cultures</td>
</tr>
<tr>
<td>Non-thermal processes</td>
<td></td>
<td>High pressure; irradiation; pulsed light; ultrasound; etc.</td>
</tr>
</tbody>
</table>
Antimicrobials in Foods

- Classified as “preservatives”
- Definitions:
  - **Chemical preservatives** (21CFR 101.22(a)(5)):
    - “…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.”
    - “[P]revent or retard deterioration” can refer to both biological and chemical deterioration
  - **Antimicrobial agents** (21CFR 170.3(o)(2)):
    - “Substances used to preserve food by preventing growth of microorganisms and subsequent spoilage, including fungistats, mold and rope inhibitors,...”
Antimicrobials in Foods

- General considerations
  - **Microorganism(s) to control**
    - Desired antimicrobial spectrum of action
  - **Length of shelf-life**
  - **Characteristics of food product**
    - $a_w$, pH, Eh, temperature, package, etc.
  - **Cost-in-use** = Usage level $\times$ price
“Clean Label” Antimicrobial

- **Identity criteria:**
  - Less/minimally processed
  - Recognizable and/or easy to pronounce name (not chemical)
    - ∴ isolated ingredients at disadvantage if chemical name must be used
    - “Tis but thy name that is my enemy.” *(Romeo and Juliet, Act 2, Scene 2)*
  - Sourced from nature / not synthetic
  - Preferably multi-use ingredient (e.g., flavoring and antimicrobial)
    - Helps shortening of ingredients statements
  - Other criteria:
    - e.g., organic, country of origin, local, environmental footprint
“Clean Label” Antimicrobials

- Many currently-used antimicrobials are found in nature
  - E.g., acetic acid (vinegar), benzoic acid (cranberries), propionic acid (Swiss cheese), lactic acid (many fermented foods)

- However, even when isolated and purified from natural sources or produced by processes that could be considered natural (e.g., fermentation), the use of their chemical names in food labels may exclude them from “clean label” consideration
<table>
<thead>
<tr>
<th>Animal</th>
<th>Plant</th>
<th>Microbial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysozyme</td>
<td>Spices and essential oils</td>
<td>Bacteriocins</td>
</tr>
<tr>
<td>Lactoferrin</td>
<td>Berries</td>
<td>Fermentates</td>
</tr>
<tr>
<td>Lactoperoxidase system</td>
<td>Olives</td>
<td>Protective cultures</td>
</tr>
<tr>
<td>Chitosan</td>
<td>Hops</td>
<td>Vinegar</td>
</tr>
<tr>
<td></td>
<td>Mustard</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alliums</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lemon juice</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cherry powder</td>
<td></td>
</tr>
</tbody>
</table>
Organic Acids and Salts

- Short-chain organic acids
- Weak acids
- Antimicrobial activity is pH-dependent
  - Increases as environmental pH approaches pKa
  - When pH = pKa, half of the acid molecules are dissociated
  - At pH < pKa, more of the acid is undissociated (and thus more effective)
- In undissociated state, organic acids enter cell, dissociate and decrease cytoplasmic pH. In effort to maintain homeostasis, cellular ATP is depleted
- Organic acids are lipophilic and therefore difficult to solubilize in water phase; therefore they are more commonly added in their salt forms
## Organic Acids and Salts

### Commonly used organic acids

<table>
<thead>
<tr>
<th>Organic acid</th>
<th>MW</th>
<th>pK&lt;sub&gt;a&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic</td>
<td>60.50</td>
<td>4.75</td>
</tr>
<tr>
<td>Propionic</td>
<td>74.08</td>
<td>4.88</td>
</tr>
<tr>
<td>Lactic</td>
<td>90.08</td>
<td>3.08</td>
</tr>
<tr>
<td>Phosphoric</td>
<td>98.00</td>
<td>2.21</td>
</tr>
<tr>
<td>Sorbic</td>
<td>112.13</td>
<td>4.80</td>
</tr>
<tr>
<td>Fumaric</td>
<td>116.70</td>
<td>3.03</td>
</tr>
<tr>
<td>Benzoic</td>
<td>122.12</td>
<td>4.19</td>
</tr>
<tr>
<td>Malic</td>
<td>134.09</td>
<td>3.40</td>
</tr>
<tr>
<td>Caprylic (octanoic)</td>
<td>144.21</td>
<td>4.89</td>
</tr>
<tr>
<td>Citric</td>
<td>192.12</td>
<td>3.14</td>
</tr>
</tbody>
</table>
Organic Acids and Salts

- Combinations of organic acids (or with other antimicrobials) can sometimes be more effective than a single one.

- Commercial applications:
  - Decontamination of fresh meat or carcasses
  - Processed meats

- Lactate (usage ≈ 2–3%), sometimes in combination with diacetate, has been used effectively in processed meats for many years.

- Recently benzoate and propionate have gained popularity due to their lower cost-in-use (usage ≈ 0.2–0.3%).

- Most are approved as GRAS.
Final word:

- Although generally not consumer-friendly due to their chemical name, they constitute the basic antimicrobial components of many natural or “clean label” antimicrobial ingredients.
Lysozyme

- Muraminidase enzyme that breaks down peptidoglycan of bacterial cell walls
  - Active against gram-positive bacteria (*Clostridium, Bacillus, Micrococcus*, specific strains of *Lactobacillus* spp. and *L. monocytogenes*)
  - By itself, ineffective against gram-negative bacteria
  - Activity can be enhanced by combining with other antimicrobials

- Commercially obtained from egg white albumin and milk

- Approvals
  - FDA: affirmed GRAS; approved for direct addition to foods
  - USDA: casings; cooked RTE meat and poultry products
Lactoferrin

- Iron-binding transferrin protein
- Commercially obtained from whey protein
- Levels increased in:
  - Unmilked animals
  - Udder infection
- Effective against bacteria, fungi and viruses
- Competes with bacteria for available iron
- Approved in U.S. for decontamination of beef carcasses and parts
Lactoperoxidase system

- System components:
  - **Lactoperoxidase**: enzyme found in mammalian secretions (raw milk, saliva, colostrum, etc.)
  - **Thiocyanate**: found in secretions; derived from the diet
  - **Hydrogen peroxide**: produced by bacteria

- **LP** catalyzes reaction of thiocyanate ion with $\text{H}_2\text{O}_2$ to produce antimicrobial compound hypothiocyanate, which disrupts bacterial inner membrane

$$\text{SCN}^- + \text{H}_2\text{O}_2 \rightarrow \text{OSCN}^- + \text{H}_2\text{O}$$
Lactoperoxidase system

- Bactericidal to many gram-negatives (e.g., *E. coli* and *Salmonella*); bacteriostatic vs. gram-positives; antifungal activity
- Promoted for preservation of raw milk
- Also tested in vegetable juices, liquid whole egg and beef
- Not very effective *in vivo*; must add thiocyanate and H$_2$O$_2$ to increase effectiveness
Chitosan

- Derived from shellfish chitin via deacetylation
- Effective against bacteria, yeasts and molds, although MIC varies widely (0.01%–5.0%)
- Mode of action not totally understood
Spice Extracts and Essential Oils

- Efficacy demonstrated in extracts and essential oils obtained from various plants
  - Most active: cinnamon (cinnamaldehyde, eugenol), oregano (carvacrol), cloves (eugenol), thyme (thymol, carvacrol)
  - Also active: rosemary, sage, basil, coriander, nutmeg, lemongrass, marjoram, cumin, others

- General susceptibility
  - Fungi > gram-positives > gram-negatives
Spice Extracts and Essential Oils

- Efficacy can be affected by high protein and fat
- Efficacy favored by low $a_w$, high NaCl, organic acids
- Chemical types:
  - Phenols: eugenol, isoeugenol, carnosol, rosmanol, isorosmanol, rosmarinic acid, borneol
  - Aldehydes: cinnamaldehyde
  - Terpenes: thymol, carvacrol
  - Thiosulfinates: allicin
Berries

- Rich in antimicrobial polyphenolics and organic acids
- Cranberry, raspberry, strawberry, bilberry, cloudberry
Olives

- *Olea europae*
- Rich in biophenols, such as oleuropein and others
- Oleuropein active against gram+ and gram– bacteria, several fungi and enveloped viruses
- Mode of action not yet fully understood
Hops

- Used to impart bitterness in brewing
- Two types of bitter compounds: α-acids and β-acids
- β-acids have antimicrobial activity against *L. monocytogenes*, *Streptococcus* spp., and *E. coli*
- Work by disrupting cell membrane
Mustard

- Active component: isothiocyanates (R–N=C=S)
  - R side group = allyl, ethyl, methyl, benzyl, phenyl
  - Inhibit gram-positives, gram-negatives and fungi
  - Low flavor threshold, but effective at very low concentrations (ng/L)
  - Mechanism of action believed to be related to inhibition of sulfhydryl-containing enzymes
Alliums

- Garlic (*Allium sativum*) and onions (*Allium cepa*)
- Active component: allicin (garlic); thiopropanal-S-oxide (onion)
- Inhibit growth and toxin production by numerous microorganisms (*C. botulinum* type A, *B. cereus*, *E. coli*, *L. plantarum*, *Salmonella*, *Shigella* spp., *S. aureus*, *A. flavus*; *C. albicans*, *Saccharomyces*, *Torulopsis*)
- Mechanism of action (allicin) believed to be related to inhibition of thiol-containing enzymes
Lemon Juice

- Active components: citric acid, ascorbic acid
- Not considered a preservative
- Available as lemon juice solids and lemon powder
Cherry Powder

- Obtained from Acerola cherries
- Active components: malic acid, ascorbic acid
- Not considered a preservative
- Primary use in meat products is as replacement for erythorbate or ascorbate
Vinegar

- Active component: acetic acid
- Very significant antimicrobial activity, but not considered a preservative
- Available in liquid and powdered form
- Buffered vinegar typically used to mitigate effects on product pH
- Popular in meat products as replacement for sodium diacetate
- Quintessential pantry ingredient → very “label-friendly”
Bacteriocins

- Heat-stable antimicrobial peptides produced by bacteria (lactic acid bacteria and others)

- Advantages
  - Can be used when fermentation conditions are not desired
  - Permit more exact dosification
Bacteriocins

- Nisin
  - Class I bacteriocin (lantabiotic) produced by *Lactococcus lactis* subsp. *lactis*
  - Inhibits growth of wide number of gram-positives as well as germination of spores of *Clostridium* and *Bacillus*
  - Inhibits cell wall biosynthesis and forms pores in cytoplasmic membrane, leading to disruption of proton motive force
  - First bacteriocin approved for food use
  - GRAS status in U.S.; approved in pasteurized process cheese, cooked RTE meat & poultry products & soups, egg products
Bacteriocins

- **Natamycin**
  - Antimycotic produced by *Streptomyces natalensis*
  - Affects cell membrane permeability, resulting in leakage of ions and peptides and, ultimately, cell lysis
  - Approved in U.S. as mold inhibitor in cheese

- **Other bacteriocins**
  - e.g., pediocin, lacticin, lactocin, lactococcin, leuconocin, sakacin
  - LAB bacteriocins are of special interest due to potential GRAS status
Fermentates

- Products of fermentation of ingredients such as sugar, dextrose, milk, whey, by lactic acid bacteria
- Active components: organic acids (e.g., lactic, propionic, acetic) and/or bacteriocins
- Activity dependent on fermenting culture used
- Many commercial preparations for meat & poultry products consists of blends with vinegar
Protective (antagonistic) cultures

- LAB (primarily *Lactobacillus* spp.) or other bacteria (primarily *Bifidobacterium* spp.)

- Work by:
  - Production of antibacterial components such as bacteriocins, enzymes, organic acids, diacetyl, ethanol, free fatty acids, H$_2$O$_2$
  - Outcompeting other microorganisms

- As opposed to fermentates, production of active component(s) takes place in the food

- Generally GRAS
Current Challenges of “Clean Label” Antimicrobials

- **Cost-in-use**
  - Generally higher than synthetic
  - Must weigh CIU vs. need/desirability of ingredient

- **Toxicological/allergenic aspects**
  - Natural ≠ Safe
  - How safe are they at levels required for antimicrobial activity?

- **Activity validation and predictive modeling**
  - Valid results require consistent concentration of antimicrobial component(s)
  - Natural variations may make this difficult or impossible
Current Challenges of “Clean Label” Antimicrobials

- **Sensory effects**
  - Two main issues:
    - Lack of refinement may leave sensory-objectionable components
    - May require high usage level for antimicrobial effectiveness
  - May lead to negative sensory attributes (flavor, color, odor, texture) at concentrations necessary for desired antimicrobial activity

- **Interaction with other food components**
  - Sometimes activity level observed *in vitro* is not observed in food product
Is it approved?

Select Ingredients
References

Thank you.