Microbiology of Spices

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Part 2
Review of Basic Microbiology

Outline – Part 2

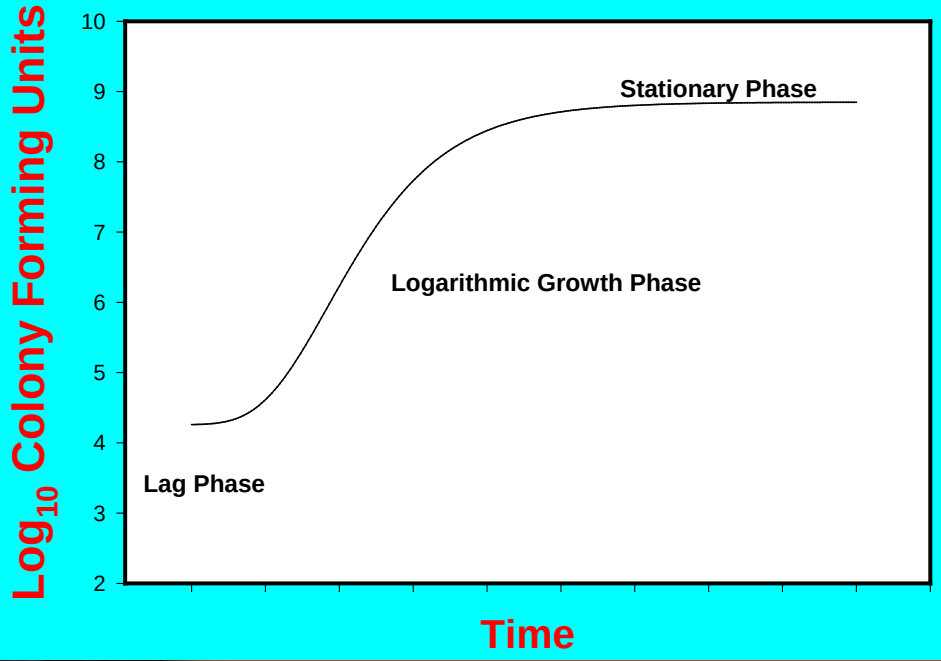
- Key Concepts in the Microbiology of Spices
- Basic outline of microbial testing methods
- Interventions
- Verification and Validation

Factors Affecting Microbial Growth and Survival

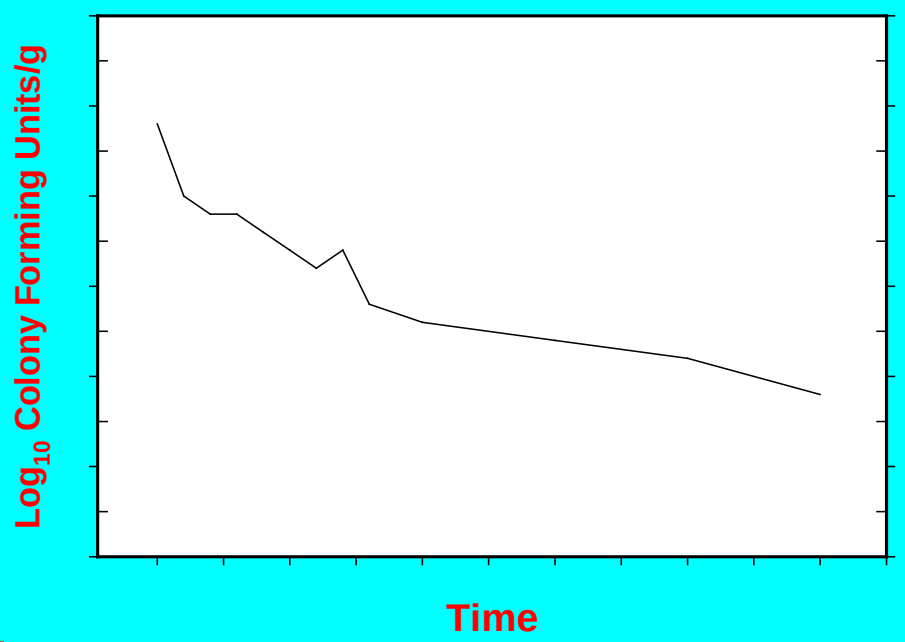
- **Source of the Microorganism**
- **Environmental conditions to allow growth or survival**
- Sufficient time for growth

Growth vs. Survival

Bacterial Growth Curve



Survival of Microorganisms



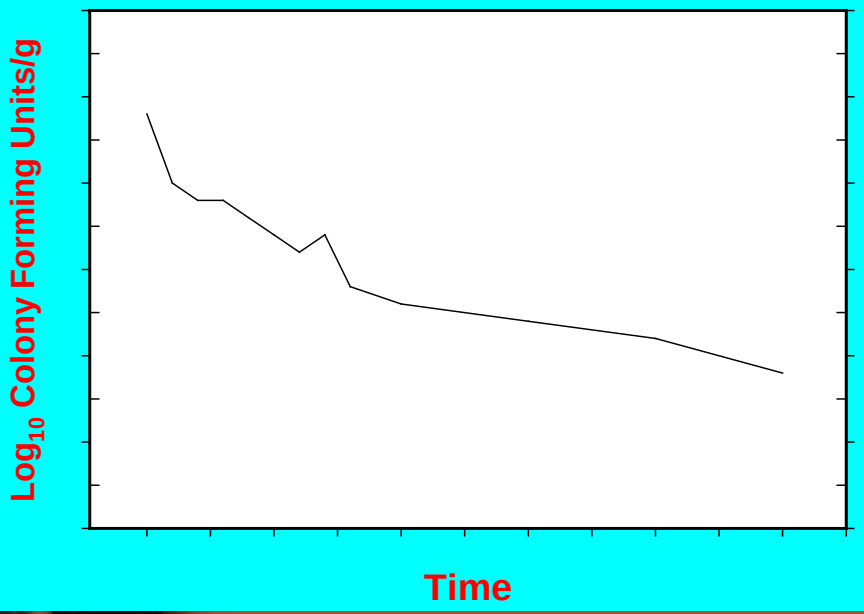
Growth vs. Survival

- Growth
  i. Lag phase
  ii. Log growth phase
  iii. Stationary Phase

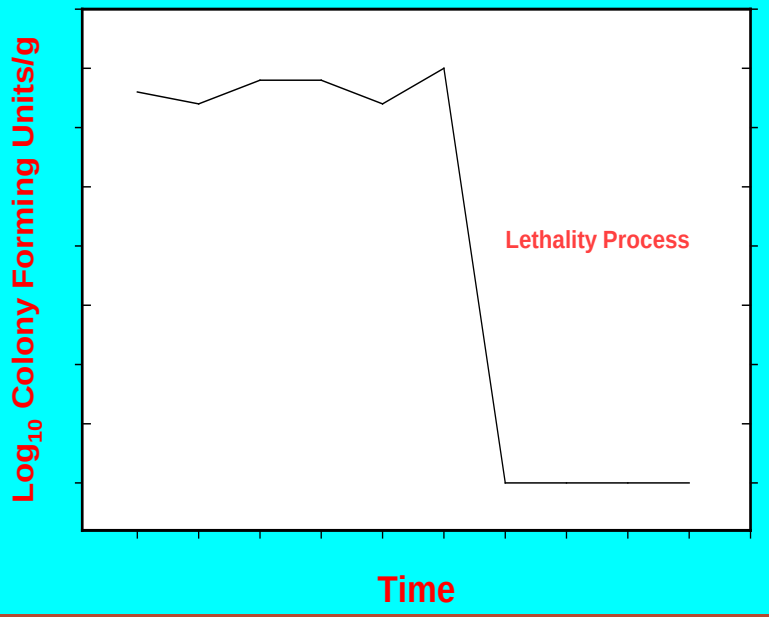
- Survival
  i. No net increase in numbers
  ii. Bacteria remain viable, but reduced metabolic activity

Survival vs. Death

Survival of Microorganisms



Application of Lethality Process



Growth vs. Survival

- Survival
  i. No net increase in numbers
  ii. Bacteria remain viable, but reduced metabolic activity
- Death
  i. Non-viable
  ii. Toxins may persist

Intrinsic and Extrinsic Parameters

- Intrinsic – inherent in the food
  - ***Water activity***
  - pH
  - *Antimicrobial properties*
- Extrinsic – environmental
  - Temperature
  - Relative humidity
  - Atmosphere

Moisture

- **Total water in a food system**:

  - **bound water** (unavailable to microbes)

  +

  - **free water** (available)

Intrinsic Parameters

- Moisture

a) Water brings nutrients into cells, contributes to pH regulation

b) Bound water – held by physical and chemical forces to marcomolecules; not available to microorganisms

c) Free water – water available to microorganisms

Moisture in Spices

- Moisture Content
- Water activity
- Relative humidity

Moisture in Spices

- Moisture Content
  - The percentage of moisture in the sample
- Usually measured by weight
  - Weigh sample
  - dry at high temperatures for a specified time
  - weigh again
- (Final weight/Initial weight) * 100

Moisture in Spices

- ***BUT*** . . . .
- Moisture content changes with environment
- High relative humidity storage conditions will increase the moisture content in spices

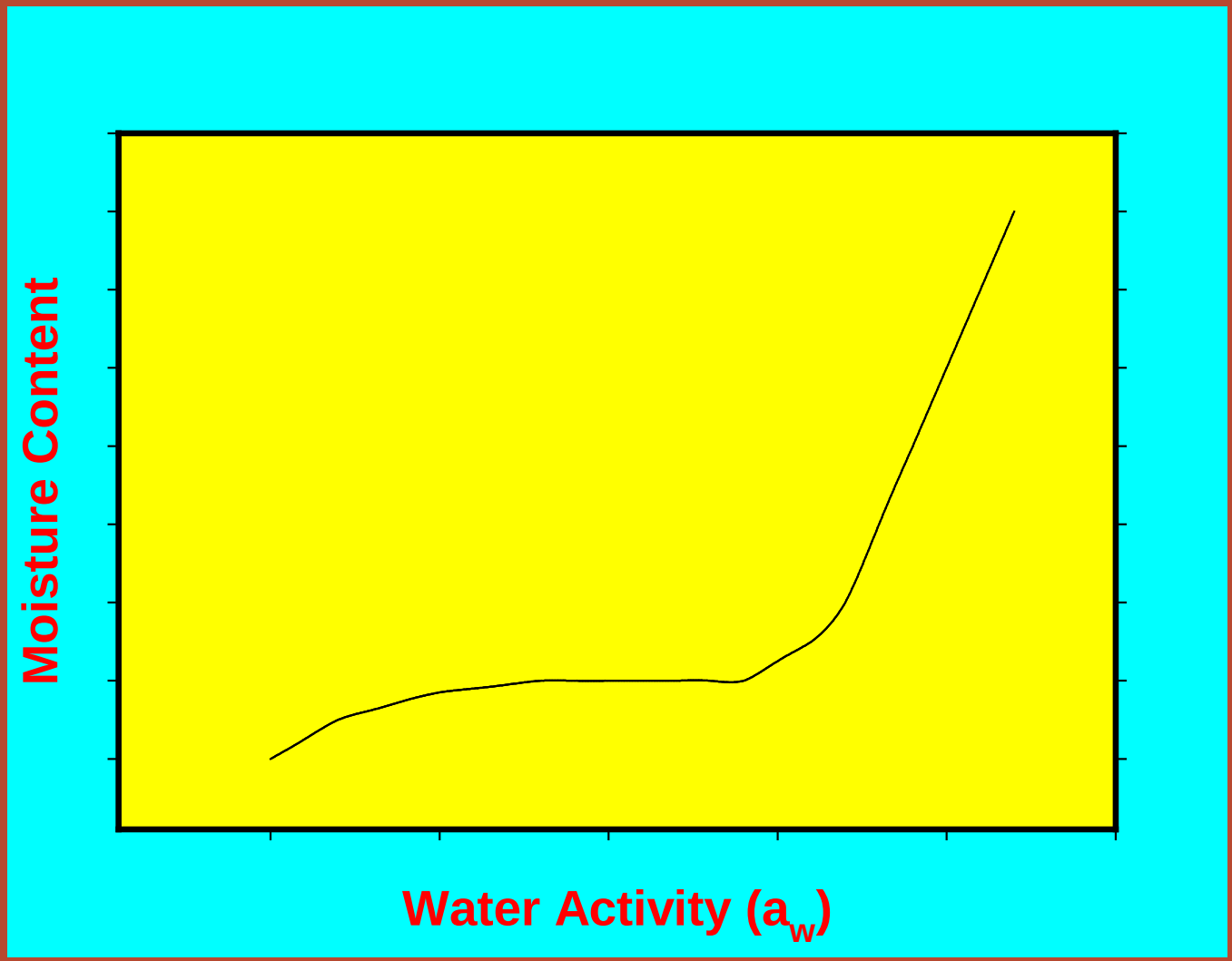
Free Water (a_w)

- Water activity (a_w)

  ratio of vapor pressure of food to vapor pressure of water at the same temperature; water a_w = 1.0

Microorganisms have minimum a_w's for growth

Relationship between moisture content and water activity



Free Water (a_w)

a_w	Microorganism	Food
0.98 – 1.0	Bacteria, yeasts, fungi	Meat, milk, fruits, vegetables
0.93 – 0.98	Bacteria, yeasts, fungi	Evaporated milk, tomato paste
0.85 – 0.93	Bacteria (some), yeasts, fungi	Cheese, cured meats
0.6 – 0.85	yeasts, fungi	Raisins, jams, syrup

Most bacteria of public health concern will survive at lower a_w's, often for extended periods of time.

Typical Water activity of Spices

Spice	Water Activity
Black Pepper 0.409 12%	0.409
Onion Powder	0.351
Cayenne Pepper	0.435
Cinnamon	0.587
Garlic Salt	0.413

Effects of low a_w on microbes

- increase in **lag phase** of growth
- decrease in **growth rate**
- decrease in size of **final population**

Examples

- High Humidity
  - Mold growth
  - Loss of quality
  - Impact on interventions
- Wet Conditions
  - Possible bacterial growth
  - Extended drying

Intrinsic Parameters

- Inhibitory Compounds (naturally occurring)

a) Naturally produced by bacteria - Colicins, bacteriocins,

b) Essential oils in spices

c) Enzymes – lactoferrin, lysozyme, lactoperoxidase system

Bacterial Stress/sub-lethal injury

- Most food processes (heat, freezing, acidic pH, inhibitors) can result in sub-lethal injury

- Sub-lethally injured cells *may* still be infective or capable of producing toxins

Bacterial Stress/sub-lethal injury

- Sub-lethally injured cells

a) Have longer lag phases

b) May have transient or permanent adaptations to additional environmental stress

c) May become sensitive to selective agents in microbiological media (may become difficult to detect using conventional methods)

Bacterial Stress/sub-lethal injury

Environmental Stress	Site of Injury
Freezing, Drying	LPS (Gram -)
Freezing, Drying, Heat	Cytoplasmic membrane
Freezing, Drying, Heat	rRNA
Freezing, Drying, Heat, Irradiation	DNA (mutations)
Freezing, Drying, Heat, low pH	enzymes

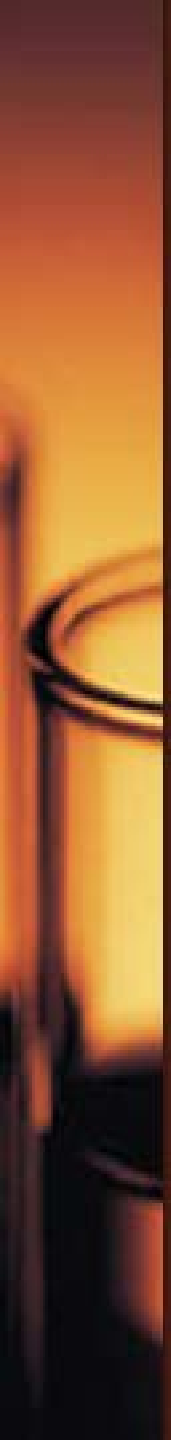
Bacterial Stress/sub-lethal injury

- Cellular Repair

a) Occurs without cell wall synthesis

b) No increase in cell numbers

c) Reducing agents (naturally occurring in food) aid repair mechanisms



MICROBIOLOGICAL METHODS

Methods to Detect Microorganisms in Foods

- Quantitative

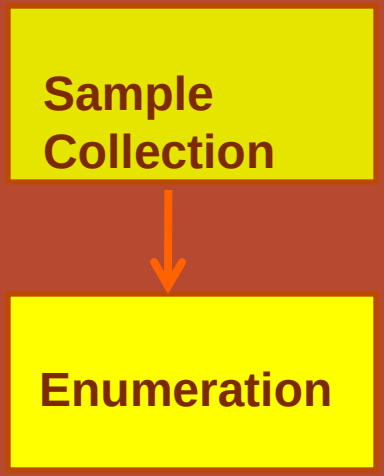
  some regulatory standards are based on quantitative measures (e.g. population of bacteria allowed in raw milk; *E. coli* Biotype I on carcasses)

- Qualitative

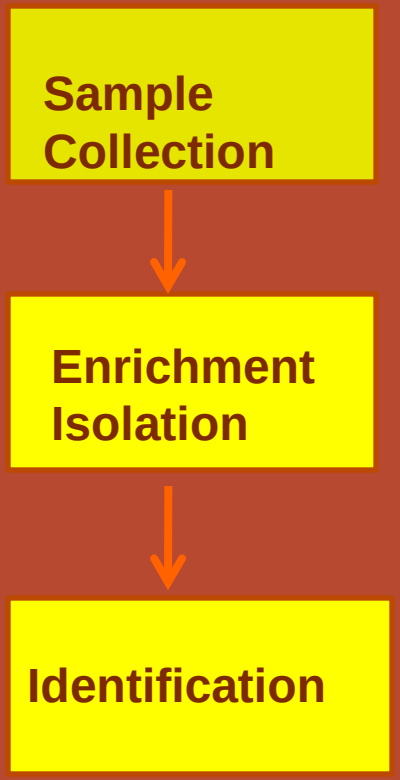
  some regulatory standards are based on qualitative measures (e.g. presence/absence of *E. coli* allowed in pasteurized milk)

Laboratory Procedures

Quantitative



Qualitative



Qualitative

- Usually pathogen
  - Salmonella
  - E coli
  - Listeria
  - Toxins (semi-quantitative)
- Food Samples
- Environmental Samples

Qualitative

- Regulatory Requirements
- “zero tolerance”
  - Salmonella in retail spices
- Sample enrichment required
- Looking for a single cell in a regulatory sample
  - 25 gr
  - 375 gr

Qualitative

- ELISA – enzyme linked immuno-sorbent assay
- PCR
- Other immunological

Qualitative Methods

- Most food borne pathogen analyses include:
  a) Non-selective enrichment (18 – 24 h)
  b) Selective enrichment (18-24 h)
  c) Detection (selective plating, ELISA, PCR)

Quantitative

- Often related to quality
- Total aerobic population
  - Pre-operational swabs
  - Shelf life determination
  - Overall Hygiene of food
- Coliforms/Enterobacteriaceae
  - Hygiene
  - Process Control Indicator

Quantitative

- Direct plating
- Most Probable Number
- “technology based”
  - impedance
  - turbidity
  - quantitative PCR

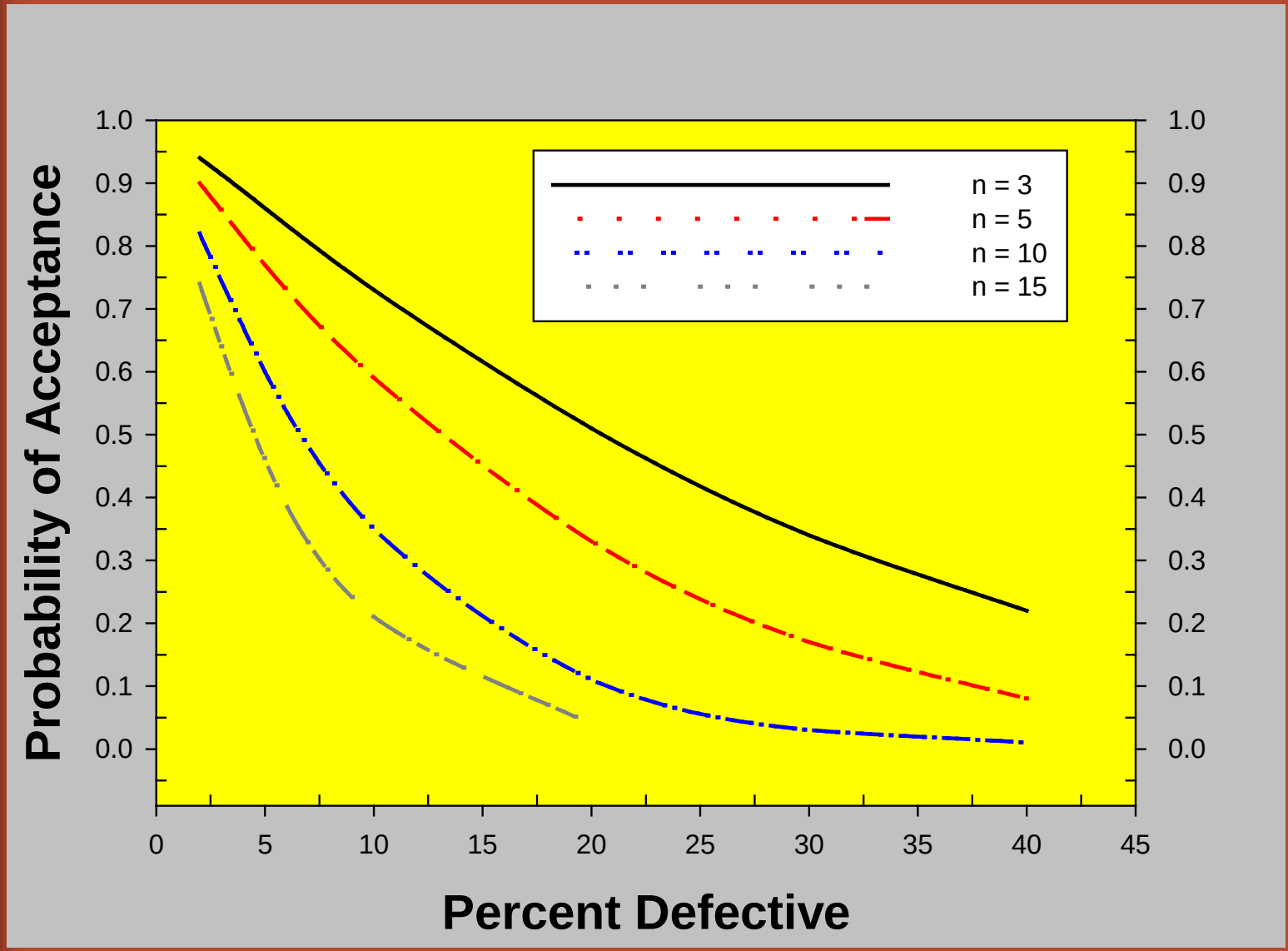
Food Samples

- Sample collection
- Sample transport
- (enrichment)
- Detection/enumeration
- confirmation

Sample Collection

- What sample?
- Statistical sampling

Operating Characteristic Curves



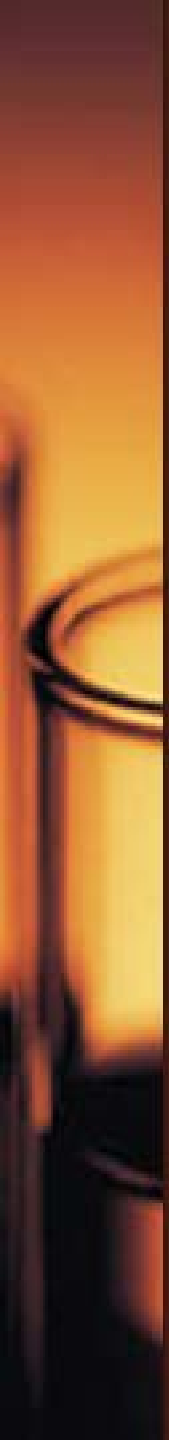
Sampling

- Sampling
  - Necessary and Appropriate
  - Demonstrates due diligence
  - Regulatory Requirement

INTERVENTIONS

Interventions

- Mechanical
- Physical Methods
  - Steam, heat
- Chemical Methods
  - Gas (ETO or PPO)
- Radiological
  - irradiation



VERIFICATION AND VALIDATION

Verification

- **Those activities, other than monitoring, that determine the validity of the HACCP plan and that the system is operating according to the plan. (NACMCF)**

Validation

- **Validation involves *obtaining evidence* that control measures, if properly implemented, are capable of controlling the identified hazards. (Codex)**

Comparison of Terms (ICMSF)

- Monitoring
  - On-going collection of information on a control measure at the time the control measure is applied.
- Verification
  - Determination that the control measures have been appropriately implemented.
- Validation
  - Collection and evaluation of scientific, technical and observational information.

Upcoming Webinars

- Webinar III
  - Interventions to control microorganisms in spices
- Webinar IV
  - Sampling and analysis