Lesson 2

The Microbiology of Food Preservation

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1. Factors affecting the growth and survival of microorganisms in foods

2. Methods of food preservation

1. Factors affecting the growth and survival of microorganisms in foods

1.1 Types of Factors

- *Intrinsec* (nutrients, growth stimulants, inhibitors, a_w, pH, Eh)
- *Extrinsic* (T, relative humidity, gaseous atmosphere)
- *Implicit* (properties the microorganisms, interacctions)
- *Processing* (elaboration, manipulation, storage)

1.2 Intrinsic Factors

1.2.1. Nutrient content

1.2.2. Antimicrobial barriers

1.2.3. Water activity

1.2.4. pH

1.2.5. Red-ox potential

1.2.1 Nutrient content

Influence of the nutrient content

Proteins, Lipids, Minerals Carbohydrates Complex

Growth factors: Additional organic nutrients

Aminoacids

Purines, Pyrimidines

Vitamines

Influence of nutrient concentration

$$\mu = \mu_{max} S / (S + Ks); \quad Ks = 10^{-5}M$$

Excep.: bottled drinking water, egg white…

Influence of other factors

- **Implicit factors**

    Prevalence of "the best"

    Growth of other microorganisms

        Biochemical modifications

        Hydrolysis of complex nutrients (proteins, lipids, polysaccharides)

            Extracellular hydrolases

            Intracelullar hydrolases (released after lysis)

- **Temperature** Lower T → More demanding :

    - inefficient transport (↑Ks)

    - enzyme synthesis reduction

To highlight

- Nutrient content does not prevent growth
- Concentration has little relevance
- Prevalence of the more efficient
- Secondary growth

1.2.2 Antimicrobial Barriers

1st

Physical barriers

2nd

Phytoalexins, Thiosulfinates, Isothiocyanates

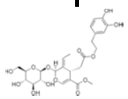
(Faseoline) (Alicine) (Mustard oil)

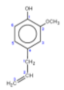
3rd

VEGETABLE FOOD

Pigments, alkaloids, resins, essential oils, organic acids

Oleuropein Eugenol Citric acid





ANIMAL FOOD

•Lactenins

•Lysozyme

•Ovoflavoprotein

•Ovotransferrin y Lactoferrin

Limitations

Low concentration

Narrow range (lysozyme)

Acción débil (spices)

Resistant strains (humulones)

1.2.3 Water Activity

- **Water availability**

$$a_w = P_v / P_v^o$$

↓

"Ratio of vapour pressure of the food to that of pure water at the same T"

Measures the amount of water available for biological activity: $0 < a_w < 1$

- **Osmotic pressure**: the pressure which needs to be applied to prevent the inward flow of water
  - Osmolysis (Cell wall, contractile vacuoles)
  - Plasmolysis (Organic compatible solutes, Ions)

1.2.4 pH

High acid food: pH $< 4{,}5$ (> 3) (Fruits, juices, fermented food)

Low acid foods: pH $> 4{,}5$ (< 7) (Vegetables, meat, fish, milk)

Range of pH for microbial growth

Pathogenic bacteria: pH > 4,5 (Excep. acid-tolerant strains, acid-adapted cells)

Acid pH

Reinforces the effect of T, a_w and preservatives

Presence of organic acids

Weak acids	>	Strong acids
Liposoluble	>	Hydrosoluble
(Acetic, Lactic, Propionic)		(Citric, Tartaric, Phosphoric)

Alkaline pH

Surface decontamination

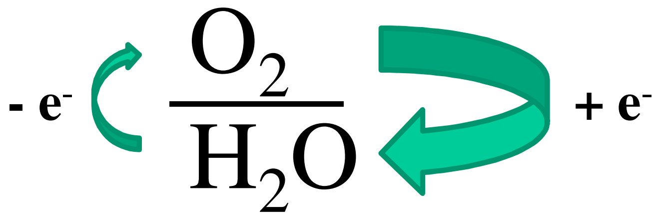
White egg, clams (≈ 8)

1.2.5 Redox Potential

Eh (mV): Measure the oxidative / reductive capacity

Oxidative Eh > 0

Reductive Eh < 0



Factors affecting Eh of food

- Redox couples present (standard redox potential; E_o)
- Ratio oxidant/reducer

$$Eh = E_o + (RT / nF) \times \ln ([Oxid.] [H+] / [Reduc.])$$

Nernst equation

- pH

	Eh	pH
Lemon	+ 383	2,2
Grape	+ 409	3,9
Pear	+ 436	4,2

Oxigen availability: Storage (Eh in air > vacuum, CO_2, N_2)
Processing (Mincing, chopping, grounding)

	Eh	pH
Wheat	-340	6
Wheat flour	+225	7
Steak	-200	5,7
Minced beef	+225	5,9

Requirement of Eh for microbial growth

Strict Aerobes**	+300 a +500 mV
Facultatives****	+100 a +300 mV
Anaerobes*	- 250 a +100 mV

1.3. Extrinsic Factors

1.3.1 Gaseous Atmosphere

1.3.2 Temperature

1.3.3 Relative Humidity

1.3.1. Gaseous Atmosphere

- **O_2**: Toxicity effect in itself > due to Eh increase

  e.g. *Clostridium acetobutylicum*

  - O_2 growth up to +350 mV

  + O_2 growth up to +100 mM

- **CO_2**: Effect on growth in itself > substitution of O_2

  - Stimulates growth of CAPNOPHILES *
  - Microbiostatic (Bacteriolytic)

1.3.2. Temperature

Effect of Temperature on microbial growth

Growth range: ↑10°C ⇒ $V_{metabol}$ X 2

Minimum T: Generation time > 10^3 min (spoilage after 3 weeks)

T < minimum: may kill slowly

Maximum T: $\mu = 0$

T > maximum: kills quickly

1.3.3. Relative Humidity

Affects a_w of food:

- Slow equilibrium between food and surrounding atmosphere
- Surface condensation → Growth of Xerotolerant → a_w Increase → ↑ Microbial growth

1.4. Implicit factors

- **Specific growth rate**

  The fastest win: Bacteria > Fungi

- **Capacity of adaptation/survival: Physiological state/Previous experience**

  Growth phase

  Pre-adaptation: Pre-exposure to stress → > Virulence

- **Interaction among microorganisms**

  Removal or production of inhibitors, nutrients (polymer hydrolysis..)

  Changes in pH, Eh, a_w, $[O_2]$…

  Intercellular communication: Induction of stress response in unstressed cells

Concluding remarks

- The physicochemical parameters determine which microorganisms can grow/prevail

- These parameters may change (processing, storage, microbial growth)

- Knowledge of food features will allow predicting
  - Identity of prevalent microorganisms
  - The possibility of food spoilage/poisoning

- It is possible to control growth and survival of microorganisms in food

- Combinations of preservation methods may allow a proper ratio security/quality

2. Methods of food preservation

2.1 Heat processing
2.2 Low-temperature storage
2.3 Control of water activity
2.4 Chemical preservatives
2.5 Modification of atmosphere
2.6 Irradiation
2.7 Pascalization

2.1 Heat processing

2.1.1 Quantifying the thermal death of microorganisms

2.1.2 Heat processing procedures

A) Pasteurization

B) Appertization

Premise: Rate of food-quality change < Rate of microorganisms death

Treatment must be adjusted to each food to:

- Destroy particular microorganisms, enzymes or toxins
- Maintain the nutritional and organoleptic quality

2.1.1. Quantifying the thermal death of microorganisms

The rate of thermal death is constant

D_T: decimal reduction time (min) at a temperature T

$$D_T = t / (\log x - \log y)$$

x = cfu before exposure to a temperature T

y = cfu before exposure to a temperature T for t min

> Initial count → > time of treatment

→ > time to sterilize

Meaning and aplications of D_T

• Establish the relative heat resistance of the microorganism

• Predict the heating time required to decrease the population to X

Example: Sample containing 10^n cfu

Heat treatment = nD_T ➡ 1 survivor

The thermal-death time curve

D value decreases as Temperature increases

$$Z = (T_2 - T_1) / (\log D_1 - \log D_2)$$

✓ Z value: number of degrees it takes to change the D value by a factor of 10

Z value depends on the microorganism

e.g. Z=16 ; D_{100}= 100 min ⟹ D_{116}= 10 min; D_{132}= 1 min

2.1.2 Heat processing procedures

a) Low temperature processes: <u>Pasteurization</u> < 100^{o}C (60-80^{o}C)

Destruction of thermosensitive microorganisms (some enzymes)

b) High temperature processes: <u>Appertization</u> ≥ 100^{o}C

“Survivors are non-pathogenic and incapable of developing within the product”

A) Pasteurization

✓ Thermosensitive food (“minimal” treatment)

✓ Requires additional treatments (thermodurics survive)

- Cooling/Freezing***
- Low pH
- Modified-atmosphere packaging
- a_w reduction
- Addition of preservatives

Pasteurization processes

- **Toxins and heat-resistant enzymes**: > 90^{o}C / 30 min
- **Milk** (*Coxiella burnetti*): 62^{o}C / 30 min (**LTLT**: Low Temperature Long Time)

  71^{o}C / 15 sec (**HTST**: High Temperature Short Time)
- **Ice cream**: 82^{o}C / 25 sec or 71^{o}C / 30 min
- **Liquid egg** (*Salmonella*): 60^{o}C / 3,5 min
- **Juice:** 60-70^{o}C / 15 min or 80-85^{o}C / 1min
- **Wine**: 82-85^{o}C / 1 min
- **Acid pickles**: 74^{o}C / 15 min
- **Vinegar**: 65-71^{o}C / 1 min or 60^{o}C / 30 min
- **Meat products**: 60-70^{o}C / variable time

HTST versus LTLT

Better nutritional and organoleptic food quality

Some enzymes resist treatment

B) High temperature processes: Appertization

Appertization: Canned + Heated = unspoiled for years under 40^{o}C

UHT : Heat-processed before packaging → Aseptic packaging

(high-pressure vapour)

Milk: 135-150^{o}C / 3-1 sec → + 3 months at < 30^{o}C

Active toxins, proteases, lipases… → Spoilage!

2.2. Low-temperature storage

2.2.1 The action of low-temperature

2.2.2. Methods of low-temperature preservation

A) Ice cooling

B) Chill storage

C) Freezing

2.2.1 The action of low-temperature

✓ **Objectives**

Prevent or reduce growth and catalytic activity

~~Killing~~

✓ **Drawbacks**

- Microbial death is not ensured
- Rate of death is unpredictable
- Does not affect endospore viability
- Chilling does not prevent endospore germination
- Raw materials must have a good microbiological (low microorganism count) quality

2.2.2 Methods of low-temperature preservation

- **Ice cooling**

Risk: Heterogeneous chilling, cross-contamination, ice melting...

- **Chill storage**

  Fruits, Vegetables: 10-20^{o}C + Humidity control

  Highly perishable: 1- 4^{o}C

  The rest of food: 4-5^{o}C

- **Freezing**

  Fast: - 78^{o}C - 196^{o}C

  Slow (storage): - 20^{o}C to - 30^{o}C

2.3 Control of water activity

2.3.1 Introduction: objectives of a_w reduction

2.3.2 Methods of a_w reduction

2.3.1 Introduction

Objectives: Prevent/reduce growth of vegetative cells (death is unpredictable)

Prevent endospore germination

Avoid toxin production

2.3.2 Methods of a_w reduction

Physical removal of liquid water

✓ **Natural dehydration**: Solar drying (Toxins)

✓ **Mechanical dehydration**

- Drying ovens or tunnels (vegetables)
- Fluidized bed (cut up vegetables)
- Hot rolls (liquids)
- Atomization (líquids)

✓ **Liofilization:** Freezing + vacuum dehydration (sublimation)

Meat, fish and other food denatured by conventional dehydration

High quality (reduce: cell injuries, protein denaturing , food browning...)

Indirect a_w reduction

✓ **Addition of salts, saccharides**

✓ **Freezing**

2.4. Chemical preservatives

2.4.1 Introduction

2.4.2 Objectives of chemical preservation

2.4.3 Allowed food preservatives

2.4.4 Smoked food

2.4.1. Introduction

Chemical compounds that kill microorganisms or control microbial growth

> 1900 : Additives (Safe?) GRAS: Generally Recognized As Safe

“Safe” chemical preservatives (47): E2_ _

Allergies, asthma, lysis of erythrocyte, vitamins loss, cancer, digestive problems, cancer...

Cumulative effects, interactions....?

2.4.2 Objectives of chemical preservation

Kill, prevent, or delay growth

(Control of low levels of contamination)

- Germicide
- Fungi (-cides, - statics)
- Viri (-cides, - statics)
- Sporo (-cides, - statics)
- Bacteri (-cides, - ostatics)

Transitory Activity

2.4.3 Allowed food preservatives

- **Nitrites**

Use: Heat-processed meat and fish (avoid growth of *C.botulinum* and toxin production)

Cheese (prevent production of gas by other clostridia)

Problem: Nitrosamine production (carcinogenic) in bacon

- **Sulfur-derived compounds**

Use: Soft fruits, juice, wine, sausages, shrimp, pickles...

Inhibits Gram-negative bacilli, moulds, yeasts (some are resistant)

Avoids browning

Problems: Produce allergies, destroy vitamin B1 in meat

- **Diacetyl**

Use: Inhibitor of bacteria (Gram-negative**; Gram-positives*)

Limitations: Aromatic, volatile, turns into acetoin (limited antimicrobial effect)

• **Bacteriocins**

Antibacterial peptides

Use: In combination (Nisin + Pediocin); food subjected to limited heat-processes (e.g. meat)

Pros: Heat resistant, stable at low T, resistant to organic solvents, bactericide at low concentration, fast action.

Cons: Sensitive to proteases, narrow range (effective against some Gram-positive and a few Gram-negative, inefficient against endospores)

Spices (effective?)

Cinnamic aldehyde (cinnamon)

Eugenol (clove and cinnamon)

Thymol (thymus)

"Certain spices stimulate microbial metabolism"

1. Garlic
2. Onion
3. Allspice
4. Oregano
5. Thymus
6. Cinnamon
7. Tarragon
8. Cumin
9. Clove
10. Lemon grass
11. Laurel
12. Chilli
13. Rosemary
14. Marjoram
15. Mustard
16. Caraway
17. Mint
18. Sage
19. Fennel
20. Coriander
21. Dill
22. Walnut
23. Basil
24. Parsley
25. Cardamom
26. Pepper
27. Ginger
28. Anise seeds
29. Celery seeds
30. Lemon/Lime

- **Acids**

Present in the raw material (citric, sorbic, benzoic)

Produced in fermentation (lactic, acetic, propionic)

Added (all the above mentioned)

<u>Objectives</u>: Antimicrobial

Taste

2.4.4 Smoked food

(Heat + Drying + Antimicrobials)

Methods: Burning wood, liquid smoke (less efficient)

Use Meat, fish, cheese

Objectives:

1.- Flavour, taste, colour, texture

2.- Increase food shelf-life

Action:

Bacteriostatic or bacteriolytic (depending on T, a_w, time of processing)

Fungicidal $^{-}$

Does not affect viability of germination of endospores

Problem: Smoke contains carcinogenic compounds

2.5. Modification of atmosphere

Combined with refrigeration

Control of fast-growing strict aerobes (non-pathogenic)

✓ Methods

– Vacuum packing: CO_2 ↑ , O_2 ↓ (meat shelf-live x 5)

– Modified atmosphere packing: $CO_2 + N_2 + O_2$

– Controlled-atmosphere storage: 10-100% CO_2
(to inhibit moulds in fruits and vegetables)

2.6. Irradiation

2.6.1. Introduction

2.6.2. Preservation with ionizing radiation

2.6.3. Efficiency of ionizing radiation

2.6.4. Dose of ionizing radiation

2.6.5. Effects of ionizing radiation

2.6.1. Introducción

High frequency/energy radiations

UV

Ionizing radiation

- **γ rays (electromagnetic radiation**, **^{60}Co,** ^{137}Cs**)**
  - penetrating (40cm)
  - cheap
  - effective

2.6.2 Preservation with ionizing radiation

- Allowed at low dose in > 40 countries

  Meat, fish, vegetable, fruit, grain.. (<u>spices</u>)

- Labelling is required by law:

  "Irradiated"

  "Processed with ionizing radiation"

- Little utilized (declared): consumer rejection (disinformation)

2.6.3 Efficiency of ionizing radiation

✓ **Time and Intensity** (power, distance)

✓ **Penetration power**

40 cm (paper, plastic, metal..)

✓ **Number and type of microorganisms**

Logarithmic death → Depends on initial microbial load → D values

> Size, complexity, and H_2O content > death

2.6.4 Dose of ionizing radiation

1 Gray (Gy) = 1 kg of food absorbs 1 joule of energy

Lethal dose

Insects	< 1 kGy
Moulds, Yeast, Bacteria	0,5-10 kGy
Endospores	10-50 kGy
Viruses	10-200 kGy

Dose allowed

Control of insects (grain, fruit), parasites (meat, fish) and germination	< 1 kGy
Control of pathogenic and spoilage microorganisms in refrigerated food (endospores and viruses excluded)	1-10 kGy
Spices and vegetable seasonings	> 10 kGy

2.6.5 Effects of ionizing radiation

Sterilizing dose: adverse side effects (colour, odour, taste, flavour, nutrients...

"Safe" dose: < 10 kGy

Pros: Penetration is deep, instant and uniform

Can be applied to packed food

Minimum reduction of organoleptic quality

Delays germination and maturation of vegetables

Cheap **Effective** **Safe** (40 years of intense studies) { Non radioactive / Non pathogenic }

Cons: Endospores, viruses and some enzymes and toxins are resistant

Mutants may emerge

Undetectable (fraud?)

2.7 Pascalization

Discovery: at the turn of the XX century → **Application**: 1980s

<u>Pros</u>:

Reduced negative effect on food quality (positive effects: gelification, sweet starch)

Instant and uniform effect, no matter the size

Induces endospore germination: increases sensitivity to adverse factors

<u>Use:</u>

Limited (on the rise, combined with pasteurization)

Acid food (Moulds, yeasts, NO *C. botulinum*): fruit derivative s (orange juice, guacamole.)