FE 305 FOOD MICROBIOLOGY
Microbial Growth Characteristics

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Important Bacteria in Foods

- Some of the most important bacterial species that cause spoilage in foods and cause various poisonings and diseases are given below. Some of them are particularly undesirable to be present in foods.

Bacillus:

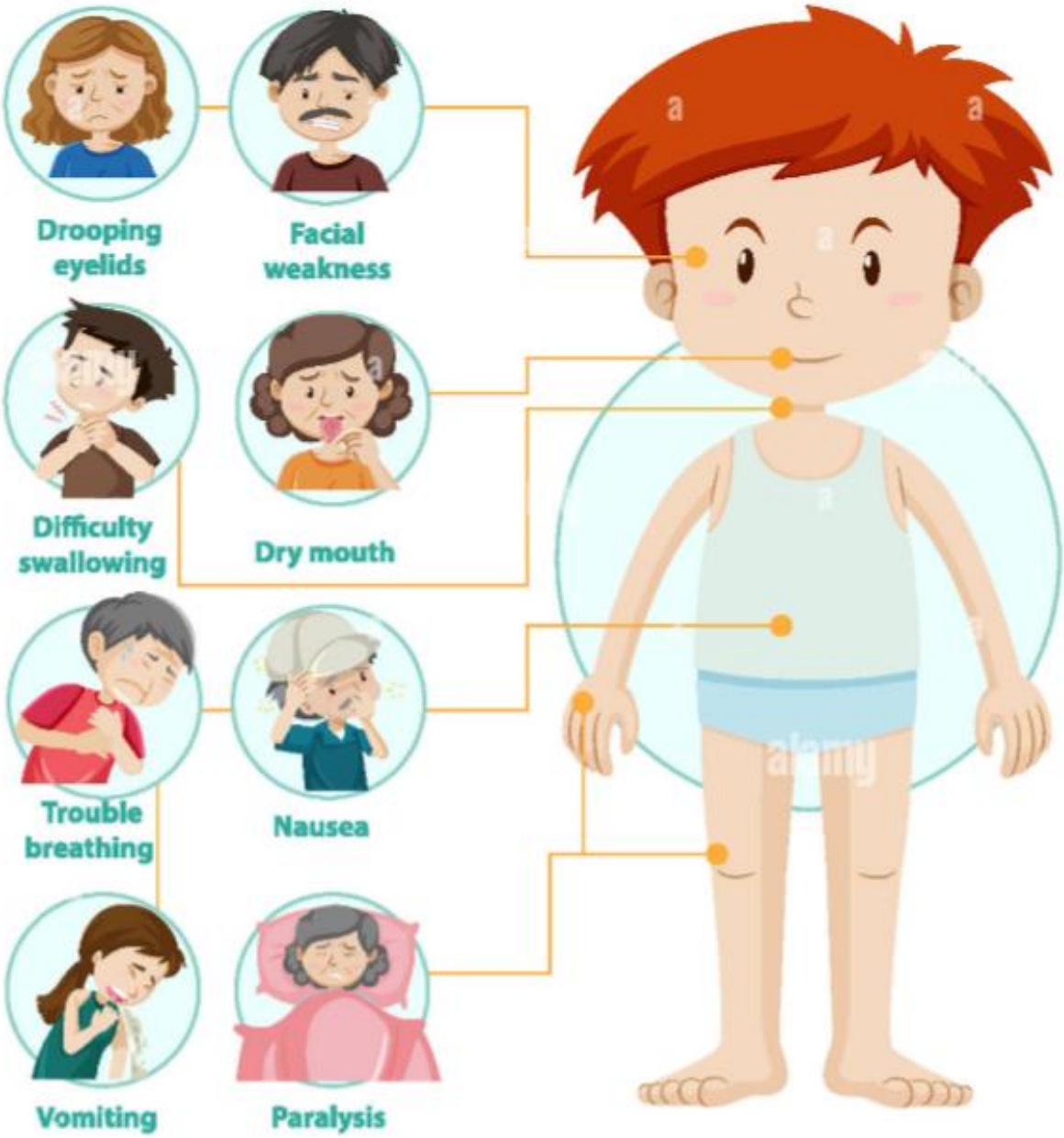
- They are mostly aerobic or facultative anaerobes, most species are motile gram-positive bacteria that can form rod-shaped endospores.
- It is commonly found in soil, water, plants, and a variety of foods.
- Most are mesophiles, but there are psychrophiles and thermophiles. Thermophilic species are especially important in the canning industry (such as *B. coagulans* and *B. stearothermophilus*) because their spores are very resistant to heat.
- *B. anthracis* is a pathogenic species that causes anthrax in humans, cattle and sheep.
- Causes poisoning by forming endotoxin in foodstuffs.
- *B. cereus* is also included in this genus.

Important Bacteria in Foods

***Clostridium*:**

- Anaerobes, gram-positive, rod-shaped, capable of producing thermostable endospores and most motile.
- It is found widely in nature in soil, water and intestinal tracts of humans.
- *C. botulinum* grows in low-acid canned foods and causes the poisoning known as botulism. It is important because it is much more lethal than other bacterial toxins.
- Bacteria in this group decompose proteins and carbohydrates and cause spoilage in foods by forming butyric acid, acetic acid, ethyl alcohol, CO_2 and H_2.
- They cause swelling (bombardment) in canned foods (such as *C. butyricum*).
- *C. perfringens* causes gas gangrene by developing in dirty wounds and food poisoning by developing especially in meat products.

BOTULISM – SYMPTOMS



Important Bacteria in Foods

***Streptococcus*:**

- They are Gram-positive, mostly facultative anaerobic bacteria.
- They form lactic acid by fermenting carbohydrates without producing gas.
- Some cause throat infections, scarlet fever and skin infections in humans and mammary infections in animals.
- Some of the lactic *Streptococcus* species are used as starters, especially in dairy products.
  - For example, *S. lactis* is used in the ripening of some cheeses.
  - *S. thermophilus* forms yogurt together with *Lactobacillus bulgaricus*.

Important Bacteria in Foods

***Escherichia*:**

- Facultative anaerobes, nonmotile or motile, short rod-shaped gram-negative bacteria.
- The most important species*, E. coli*, is naturally found in the intestinal flora of humans and animals, and then infects water, soil and other places.
- Therefore, the presence of *E. coli* in food and water is important as an indicator of fecal contamination.
- It is frequently seen in foodstuffs. These bacteria form acid and can produce gas.

***Salmonella*:**

- Gram-negative, motile, aerobic and facultative anaerobes are rod-shaped bacteria. They do not form spores.
- It is naturally found in the intestines of humans and animals and in sewage waters.
- All species are pathogenic to humans. They cause intestinal infection in humans.

Important Bacteria in Foods

***Shigella*:**

- They are gram-negative, nonmotile, facultative anaerobic and rod-shaped bacteria.
- It is found in sewage waters and in the human gut. It causes intestinal infections in humans.
- They produce acid by fermenting glucose.

***Staphylococcus*:**

- Gram-positive, aerobic or facultative aerobes are nonmotile and non-spore-shaped cocci.
- The most important species are *S. aureus* and *S. epidermidis*. Both species occur naturally in the skin and nasal flora of humans and animals.
- *S. aureus* causes abscess infections and mastitis in animals and humans.
- The disease caused by the ingestion of toxins produced by *S. aureus* strains in food is one of the most common food poisonings.

Growing of Microorganisms in Foods

- Microorganisms need nutrients to survive and reproduce.
- Foods contain components necessary for maintaining vital activities for microorganisms as well as for humans.
- Therefore, they are important areas of microbial growth.
- It is accepted that there are no microorganisms in the inner parts of the foodstuffs found in nature, that is, the inner parts are sterile.
- There is a variable variety of microorganisms on the surfaces of foods. The amount of microorganisms that can be found on the surface of the food depends on the relationship between the size of the food surface and its volume.
  - Fruits and vegetables such as cucumbers and tomatoes have a small surface compared to their volume.
  - Foods such as lettuce and parsley have a large surface according to their volume. This means more areas where microorganisms can be found and multiply.

Microbial Growth

- Microorganisms can be found in the air, water, soil and food as well as in our environment.
- Contamination of fresh foods, prepared foods and even preserved foods with microorganisms is possible.
- Uncontrolled and undesirable microbial growth in foods causes great damage to foods and causes economic losses as well as unlimited nutrient losses.
- Some microorganisms and foods with microbial contamination cause food infection and food poisoning.

Inhibiting Microorganism Growth in Foods;

1. Prevent contamination
2. Inhibit the development of infecting microorganisms
3. Neutralize or kill them

Microbial Growth

- Despite the undesirable effects, since various foods are produced by microbial activity, the microbial activity in these foods is desirable.
  - For example:
    - bread,
    - yogurt
    - cheese,
    - beer,
    - antibiotic,
    - vaccine,
    - vitamin,
    - enzyme vs.

  are necessary for its preparation.

Microbial Growth

- All chemical substances that a microorganism receives from the external environment are its nutrients.
- With the oxidation of chemical substances, energy is released and this energy is used in various activities in the cell.
- One of the activities that require energy is development/proliferation.
- Nutrients/Energy
  - Proliferation;
  - Movement,
  - regulation of activities in the cell, and
  - It is also necessary for its repair.
- Organisms that provide the necessary energy by using chemicals are called chemotrophs.
  - Chemotrophic microorganisms obtain their nutrients from organic or inorganic substances and light.
    - Examples of organic substances; carbohydrates, proteins and fats can be given.
    - Inorganic sources; hydrogen sulfide, ammonia, hydrogen, iron can be given.

Microbial Growth

- In order to survive of microorganisms;
  - Carbon (organic substances, organic acids, CO_2)
  - Nitrogen (essential for amino acids, nucleic acid synthesis: proteins, peptides, N_2, NO_3 or NO_2 are used as sources)
  - Phosphorus, sulfur, magnesium, potassium, and sodium are needed.
  - Oxygen/carbon dioxide
  - Zinc and iron from minerals are necessary for the cell.
- They provide the necessary energy in the cell by oxidizing carbohydrates.
  - Glucose is the most common energy source
  - To generate energy from glucose
    - Breathing and
    - Fermentation is used.

Microbial Growth Characteristics in Food

1) Competition

- Microorganisms compete for nutrients.
- Competition of microorganisms for limited nutrients leads to interactions between cells in population or between different microorganisms,
  - results in a reduction of growth rate of some microbial groups.
  - This may lead to slower-growing or inhibition.
- Some species are much better adapted to growth at very low substrate concentrations,
  - whereas others require higher concentration of substrates due to more specialized growth rates.
- Foods contain a mixed population of microorganisms.
- Competition among microorganisms in a food determines which one will outgrow the others and
  - cause its characteristic type of changes.

- Some microorganisms will become predominant.
- If conditions are favorable for all types of microorganisms,
  - foods are mostly spoiled by bacteria rather than by yeasts and molds
    - because bacteria have shorter generation time.
- Yeasts grow faster than molds.
- Molds can predominate only when conditions are better for them than for yeasts or bacteria.
- Thus, in a mixed population,
  - the intrinsic and extrinsic factors predict which will become predominant and
    - produce specific changes in a food.
- This is very important in the microbial spoilage, food poisonings and bioprocessing.

2) Metabiotic (Sequential) Growth

- Different types of microorganisms present in foods,
  - But one type(s) can dominate in foods.
- Initially, one or two types may grow optimally
  - inhibit others.
- But another type can find this changed environment to be favorable for growth
  - and grow rapidly.
- Sequential dominance can occur several times during the storage period of a food.
- If a food is packaged in a bag with a little air,
  - aerobes will grow first and utilize the oxygen.
  - Environment will become anaerobic,
    - anaerobes grow.

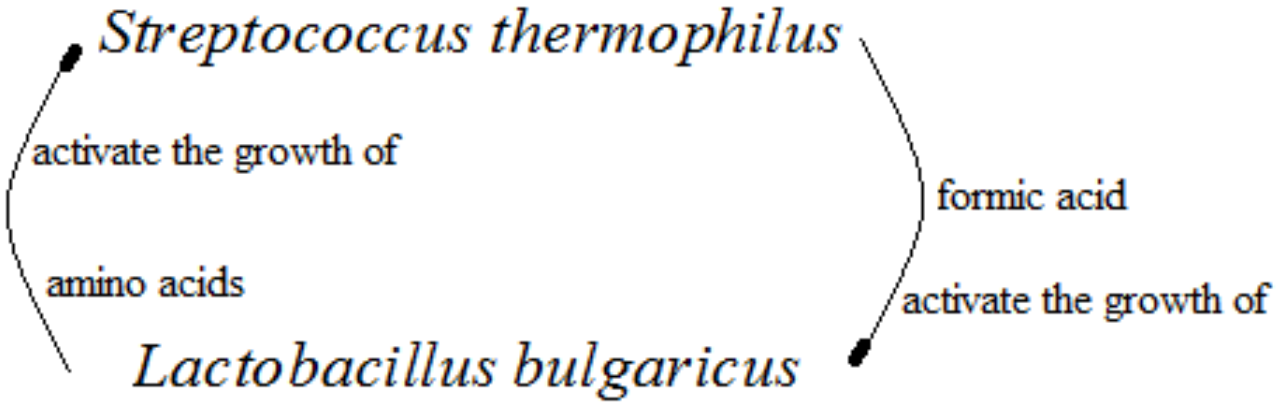
- Most natural fermentations associate with metabiosis.
- In **sauerkraut fermentation**,
  - four different bacterial species grow in succession,
  - one creating the favorable growth condition for the next one.
  - Desirable characteristics of final product depends on the growth of four species in sequence.
    - Different bacteria mainly coliforms grow first; produce acids that activate the growth of lactic acid bacteria (LAB);
    - second, *Leuconostoc mesenteroides*; to produce acids;
    - third, *Lactobacillus plantarum*; to produce acids and
    - last, acid-tolerant *Lactobacillus brevis* grow.
  - increase acidity of product in order until their growth stop.
  - These growth produce product with characteristic flavor and texture.
- If preservation methods are not used;
  - due to increase in acidity of product, film yeasts and molds grow over the top and reduce the acidity.
    - spoil product.

Another example to meatbiotic growth:

- **Raw milk spoilage** at room temperature due to fermentation
  - by coliforms first to produce acids.
  - Acids activate the growth of *Lactobacillus lactis*.
    - to produce acids.
  - Next the acid-tolerant *Lactobacillus* such as *Lb. brevis* grows.
    - increases the acidity further until they are stopped.
  - Then film yeasts and molds grow over the surface to reduce acidity.
  - Finally acid proteolytic bacteria can grow such as *Enterococcus faecalis* subsp. *liquefaciens* and *Micrococcus caseolyticus*.

3) Symbiotic Growth

- In symbiotic growth, two or more microorganisms help one another during growth in food.
- One type may produce metabolic product(s) that the second type needs for proper growth, but cannot produces itself.
- In turn the second species produces a nutrient that stimulates the first one to grow better.
- Synergism occurs between *Lactobacillus arabinosus* and *Enterococcus faecalis* in a minimum medium in which each organism is unable to grow itself.
- *E. faecalis* requires folic acid, which is produced by *Lb. arabinosus*, that requires phenylalanine, which is produced by *E. faecalis*.



4) Synergistic Growth

- When two types of microorganisms grow together
  - may able to bring changes which could not produced alone.
- When *Str. thermophilus* and *Lb. bulgaricus* grow in milk independently, they produce about 8 to 10 ppm acetaldehyde,
  - the desirable flavor component of yogurt.
- When they grow together in milk, they produce 30 ppm or more acetaldehyde.
- Another examples *Pseudomonas syncyanea* growing alone in milk produces only a light-brownish color and *Lac. lactis* does not color change in milk.
  - When two bacteria grow together,
    - a bright blue color develops.

5) Commensalisms

- Microorganisms may not affect each other
  - but one organism use the substrate which is produced by others.
  - For example, cellulose hydrolyzing microorganisms produce glucose and cellulose non-hydrolyzing organisms use glucose.
- Anaerobic thermophilic bacterium, *Clostridium thermocellum* produces complex enzymes to hydrolyze cellulosic substrates.
- Other bacteria producing cellulose hydrolyzing enzymes are *Acetivibrio cellulolyticus*, *Bacteroides cellulosolvents*, *Clostridium cellulovorans* and *Ruminococcus flavefaciens*.
- One population benefits from growth factors excreted by a population, while the latter unaffected.

- Another example to commensalism type of growth is the vinegar production.
  - In vinegar production yeasts (*S. cerevisiae*) produces alcohol from sugar and
  - *Acetobacter* (*A. aceti*) and *Gluconobacter* (*G. oxydans*) use ethanol to produce acetic acid.
- Vinegar involves the production of acetic acid.

6) Antagonistic Growth

- Microorganisms can adversely affect each other.
- Sometimes one can kill the other.
- This presents among many bacterial strains,
  - between bacteria and yeasts, between yeasts and molds, and between bacteria and molds.
  - This occurs due to the production of one or more antimicrobial components by one or more strains in the mixed population.
- Some Gram-positive bacteria produce antimicrobial bacteriocins that can kill many other types
  - such as *Lactococcus lactis* subsp. *lactis* produces nisine which inhibits Gram-negative bacteria and kills Gram-positive bacteria.
- Antagonistic characteristic is important in the control of spoilage and pathogenic microorganisms in food.
- Production of lactic acid by LAB or sulfuric acid by *Thiobacillus thiooxidans* inhibits other non-acidophilic microorganisms.

7) Predation

- It is the growth of bacteria on other microorganisms.
- *Bdellovibrio* (*Bdellovibrio bacteriovorus* in soil), *Daptobacter* and *Stenotrophomonas maltophilia* attach to Gram-negative bacteria,
  - penetrating the cell wall, and multiplying within the periplasmic space.
- They reproduce on cellular components of the host cell.
- Cell content of host is partially degraded and utilized.
- *Myxococcus* can cause lyses of susceptible strains at some distance, apparently with the aid of exoenzymes.
- *Myxococcus* derive their nutrition from material released by the lysed cells.
- These parasites differ from viruses in that
  - it does not depend directly on the metabolic activity of host cell.

Factors Effecting Microbial Growth in Foods

- There are many factors that affect the growth of microorganisms in foodstuffs.
- The effects of these factors;
  - Characteristics of the foodstuff and the environment in which the food is found,
  - The method used to process the food, and
  - It differs depending on the type of microorganism.
- Factors affecting the growth of microorganisms are also directly related to the protection of food from microbial activities.
- With these methods, hurdles have been developed for food preservation methods or processes.

Factors Effecting Microbial Growth in Foods

Intrinsic factors	Extrinsic factors	Implicit factors	Processing factors
Nutrient content pH and buffering capacity Oxidation-reduction potential Water activity (moisture) Antimicrobial constituents Biological structure	Relative humidity Storage temperature Gaseous atmosphere	Microbial alteration Synergism Metabiotic Antagonism Commensalism Growth rate	Physical treatments Use of chemicals Changes in pH Contamination

Factors Effecting Microbial Growth in Foods

1. Internal factors;
    a. Nutrients,
    b. Acidity, Hydrogen ion activity (pH),
    c. Oxidation-reduction potential (O/R),
    d. Water activity (aw),
    e. Substances found naturally in foods that increase or decrease reproduction (inhibitory) or Substances added to foods as preservatives
    f. Structure of the foodstuff
2. External factors (Environmental factors);
    a. Relative humidity,
    b. Composition and density of gases in the environment,
    c. Ambient temperature and time
    d. Radiation

Water Activity

- The survival of microorganisms and their metabolic activities depend on the presence of water in the environment. **Water activity (aw)** is defined as the amount of free water available to the microorganism. It is denoted by aw or Aw.
- The highest value of water activity is 1.0 (pure water) and the lowest value is 0.0.
- Free water in food can be removed from food by drying and freeze-drying processes.
  - For this reason, the water activity value of fresh foods is high (fresh fruit aw value: 0.95),
  - The water activity value of dried foods (dried fruit aw value: 0.60) will be low.
- In addition to drying, the water activity of the environment can be reduced by adding substances such as salt (NaCl) and sugar to the environment.
- In this way, the growth of microorganisms can be delayed or stopped.
  - This method is used in the production of meats that are dried by coating with jams, brines and salt.

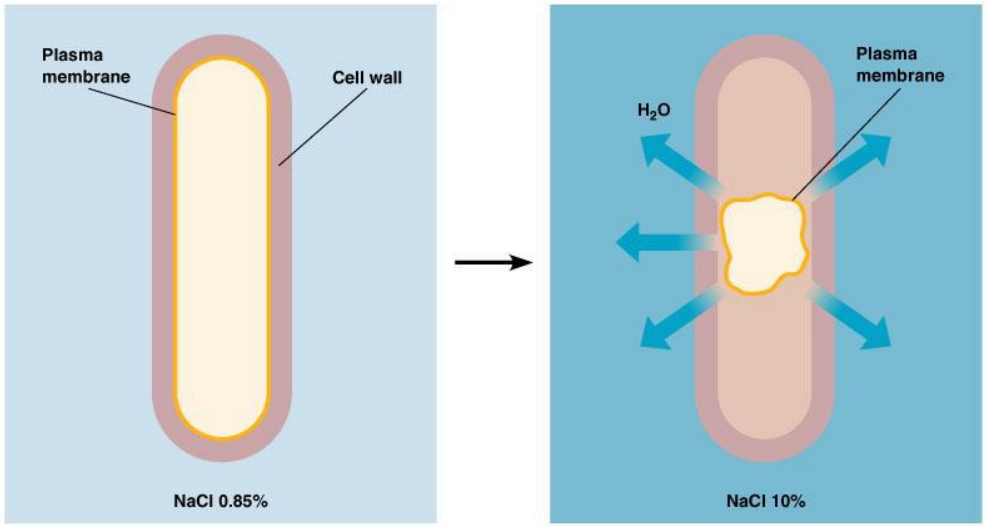
Water Activity

- Some water-binding substances (NaCl) have a direct effect on microorganisms.
- The aw value is affected by other factors
- At the optimum temperature, the microorganisms resist the lower aw value better.
- Aerobic microorganisms can grow at lower aw values in the presence of oxygen.
- In the presence of an inhibitor, it narrows the aw range in which microorganisms can grow.
- In environments with high salt concentration, water moves out of the cell
  - High salt concentration impairs bacterial metabolism
  - If not prevented, it will lead to plasmolysis.
    - Dehydration and shrinkage
    - Damage to the cell membrane

Water Activity

Classification by osmolarity

- **Halotolerant**
  - Grows at low salt concentrations but resistant to high salt concentration
- **Halophile**
  - High salt concentrations are needed
- **High Halophile**
  - Requires high salt concentration (> 4 M)
- **Osmophile**
  - Bacteria living in dry environments
  - Yeasts living in high sugar concentration



A_w	Growing of microorganisms in Relevant Value	Food Example
0.95	*E. coli, Clostridium perfringens, Many saprophytic microorganisms some yeasts*	Perishable foods (fresh and canned fruit, vegetables, meat, fish), milk, cooked sausage, bread, foods containing 4% sucrose or 7% NaCl
0.91	*Salmonella spp., C. botulinum, Lactobacillus spp., some molds*	Some cheeses, cured meats, juice concentrates, foods containing 55% sucrose or 12% NaCl
0.87	Most of the Yeast	Fermented sausages, some cakes, hard cheeses, margarine, foods containing 65% sucrose or 15% NaCl
0.80	Most of the molds *Saccharomyces spp., Staphylococcus aureus*	Most juice concentrates, condensed milk, syrup, flour, high-sugar cakes, some cured meats
0.75	Most halophilic bacteria Mycotoxigenic *Aspergillus* spp.	Jam, marmalade, fruit candies, marzipan, some biscuits, some cured meats
0.65	Some molds	Oatmeal at 10% moisture, jelly, molasses, nuts
0.60	Osmophilic yeasts and Xerophilic molds	Dried fruits15-20% moisture, caramel, marshmallows, honey
0.50	No growth	Pasta 12% moisture , 10 % moist spices
0.40		Egg powder %5 moisture
0.30		Biscuits, crackers, dried bread 3-5% moisture
0.20		Milk powder at %2-3 moisture, Dried soups

Acidity (pH)

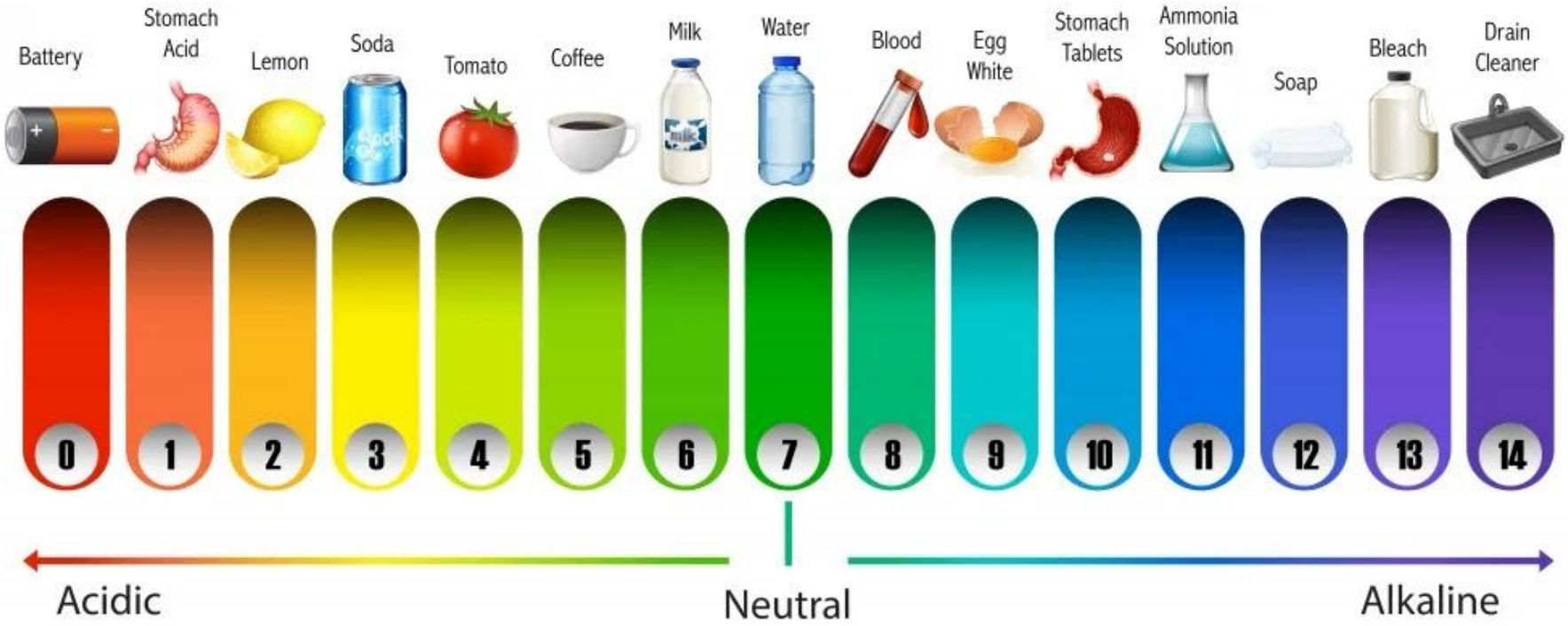
- The acidity of foods is expressed in terms of pH and total acidity. pH indicates the H^+ ion concentration in the medium and is graded between 0 and 14.
- pH has an important effect on the growth of microorganisms.
- Most microorganisms, especially pathogens, grow in a fairly narrow pH range. Even a small change in pH can stop reproduction.
- Microorganisms generally grow well at pH values close to neutral (pH 6.0 to 8.0).
  - Molds can grow between pH 1.5-11 and bacteria that can grow between pH 1-9.
- When the pH demands of microorganisms are compared, bacteria are more sensitive than molds and yeasts.
- When looking at the microbial safety of a food, foods below pH 4.4 are considered **safe food** as pathogens cannot grow.

Microorganism	Minimum pH	Optimum pH	Maximum pH
Bacteria	4.1 – 4.5	6.5 – 7.5	9.0
Yeast	1.5 – 3.5	4.0 – 6.5	8.0 – 8.5
Mold	1.5 – 3.5	4.5 – 6.8	9.0 – 11.0

Acidity (pH)

- Foodstuffs are acidified and their pH is lowered, so they can be stored for a longer period of time. A low pH value affects microorganisms in two ways:
  - It prevents the growth of microorganisms.
  - At low pH, microorganisms are more affected by heat.
- Temperatures higher than 100°C are required for sterilization of cans with a pH value above 4.5, while temperatures below 100°C are sufficient to inhibit microorganisms in cans with a pH value below 4.5.
- **Acidity** can be found in foods for three reasons:
  - It originates from the natural structure of the food.
  - It is caused by the development of microorganisms (fermentation, etc.) in food, voluntarily or involuntarily.
  - It is caused by organic acids deliberately added to food.

Acidity (pH)



- Acidic medium;
  – Mold, yeast and Lactobacilli

- Neutral medium;
  – Most of the pathogenic bacteria

- Alkali medium;
  – *Vibrio cholerae*, *Mycoplasma*, Soil bacteria

Approximate pH Values of Some Foods

CAN BE INCLUDED IN YOUR 20% ACID

Highly Alkaline

pH 9.5 alkaline water

Himalayan salt

Grasses
Cucumber
Kale
Kelp
Spinach
Parsley
Broccoli
Sprouts (soy, alfalfa etc)
Sea Vegetables (Kelp)

Green drinks

All Sprouted Beans/ Sprouts

Moderately Alkaline

Avocado
Beetroot
Capsicum/Pepper
Cabbage
Celery
Collard/Spring Greens
Endive
Garlic
Ginger
Green Beans
Lettuce
Mustard Greens
Okra
Onion
Radish
Red Onion
Rocket/Arugula
Tomato

Lemon
Lime

Butter Beans
Soy Beans
White Haricot Beans

Chia/Salba
Quinoa

Mildly Alkaline

Artichokes
Asparagus
Brussels Sprouts
Cauliflower
Carrot
Chives
Courgette/Zucchini
Leeks
New Baby Potatoes
Peas
Rhubarb
Swede
Watercress

Grapefruit
Coconut

Buckwheat
Quinoa
Spelt
Lentils
Tofu

Other Beans & Legumes
Goat & Almond Milk

Most Herbs & Spices

Avocado Oil
Coconut Oil
Flax Oil/ Udo's Oil

Neutral/ Mildly Acidic

Black Beans
Chickpeas/Garbanzos
Kidney Beans
Seitan

Cantaloupe
Currants
Fresh Dates
Nectarine
Plum
Sweet Cherry
Watermelon

Amaranth
Millet
Oats/Oatmeal
Spelt
Soybeans

Rice/Soy/Hemp Protein

Freshwater Wild Fish

Rice & Soy Milk

Brazil Nuts
Pecan Nuts
Hazel Nuts

Sunflower Oil
Grapeseed Oil

Moderately Acidic

Fresh, Natural Juice

Ketchup
Mayonnaise
Butter

Apple
Apricot
Banana
Blackberry
Blueberry
Cranberry
Grapes
Mango
Mangosteen
Orange
Peach
Papaya
Pineapple
Strawberry

Brown Rice
Oats
Rye Bread
Wheat
Wholemeal Bread
Wild Rice
Wholemeal Pasta

Ocean Fish

Highly Acidic

Alcohol
Coffee & Black Tea
Fruit Juice (Sweetened)

Cocoa
Honey
Jam
Jelly
Mustard
Miso
Rice Syrup
Soy Sauce
Vinegar
Yeast

Dried Fruit

Beef
Chicken
Eggs
Farmed Fish
Pork
Shellfish

Cheese
Dairy

Artificial Sweeteners
Syrup

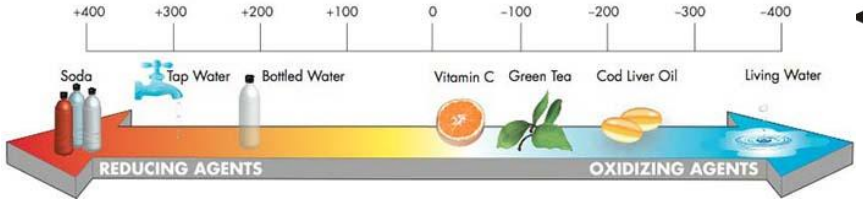
Mushroom

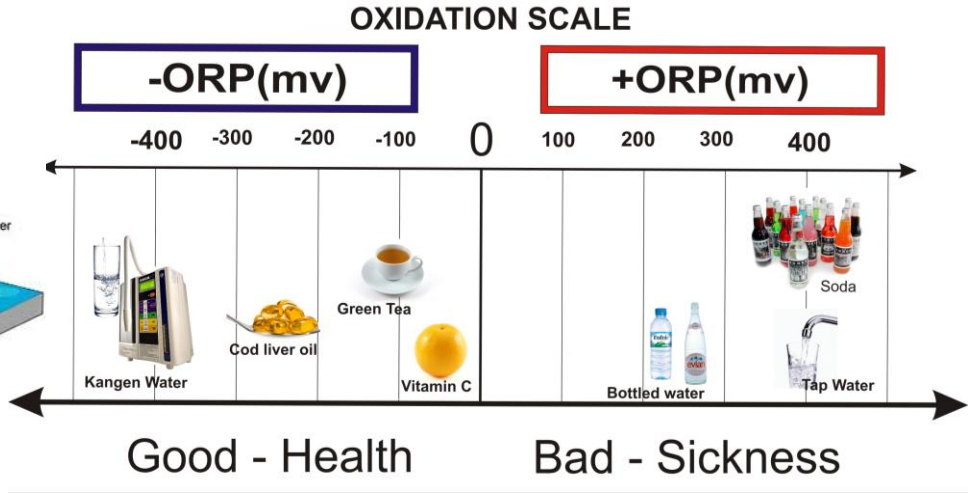
Acidity (pH)

- Since the pH values of fruits are generally below pH 4.5, their microbial deterioration is caused by yeast and molds that can develop below pH 3.5.
  - This value is well below the minimum pH values where many bacteria that cause food spoilage and pathogenic bacteria can grow.
- In products such as cheese, sausage and pickles obtained by fermentation from milk, meat and vegetables, bacteria form lactic acid and lower the pH.
  - Thus, the development of many unwanted bacteria is prevented.
- The reason why products such as various fruits, juices, soft drinks, vinegar and wine are stored for a long time without spoiling is that their pH values are below the pH value where bacteria can grow.

Oxidation-Reduction Potential

- Oxidation–reduction potential (E_h) is a measure of the ability of chemical/biochemical systems to oxidize (lose electrons) or reduce (gain electrons).
- The oxidation-reduction potential is also called the redox potential. It is denoted as O/R and Eh.
  - A positive value indicates an oxidized state, whereas a negative value indicates a reduced state. The E_h of milk is about +150 mV and that of cheese is about -250 mV.
- The redox potential indicates the relationships of microorganisms with oxygen.
  - Aerobic microorganisms, high or positive to grow;
  - Anaerobic microorganisms, on the other hand, require low or negative redox values.
- Eh values of foods vary between +500 mV and -500 mV.
- Eh values of vegetables and fruits are measured between +300 and +400 mV.
  - This means that aerobic bacteria and molds can easily grow and spoil fruits and vegetables.
- While the Eh values of large pieces of fresh meat are measured at -200 mV, the Eh value is around +200 for minced meat. From these measurement values, it can be concluded that the Eh value decreases by mixing with the air while the meat is being cut.
- The fact that the Eh values measured in canned foods vary between -20 mV and -440 mV is an indication that anaerobic microorganisms can develop in these foods.





Antimicrobial Substances

- Substances that kill microorganisms (**microbicide**), prevent their reproduction (**microbistatic**) or prevent their activity (**inhibitory**) are called **antimicrobial agents**.
  - Substances that kill bacteria are called **bactericide**, and substances that stop bacteria from multiplying are called **bacteriostatic**.
  - Substances that kill fungi (yeasts and molds) are called **fungicides**, and substances that stop the growth of fungi are called **fungistatics**.
- The use of antimicrobial substances is important to prevent unwanted microbial growth in the production of foods.
- Antimicrobial substances can be found in the natural structure of foods, as well as those that can be added externally.
- In order for antimicrobial substances to have the desired effect, the amount of use must be well adjusted and the pH of the food, water activity and other food components must be considered.
- When adding antimicrobial substances to foods, the amounts specified in the regulations and by-laws should be taken into account.

Antimicrobial Substances

Foods Containing Antimicrobial Substances Naturally in Their Structure and Antimicrobial Substances Contained in These Foods

Foods	Antimicrobial Substances	Foods	Antimicrobial Substances
Cow milk	Lactoferrine, Konglutinine, Lactoperoxidase	Cinnamon	Cinnamic aldyde and Eugenol
Egg	Lysozme	Mustard	Allyl izothyociyanade
Onion-Garlic	Allisine	Sage	Eugenol and thmol
Clove	Eugenol	Fruit, Vegetable, Tea, Molasses	Hydroxy sinnamic acid
Citrus fruits (in peel)	Citral		

Antimicrobial Substances

- Microorganisms with which some antimicrobial substances can be added to foods externally and some foods where they are used

Antimicrobial substance	Microorganisms to which it is effective	Used Food
Sulphur dioxide (SO_2) and sulfides	Mold, yeast and bacteria	Dried fruits, syrups, purees
Nitrit and nitrate	*Clostridium botulinum, Clostridium butyricum, Clostridium sporogenes*	Meat and meat products, cured fish
Sorbic acid	Mold and yeast	Cheese, cereals, jams, marmalades, sauces, margarine, ketchup, dried fruit, meat and fish products, pickles and brine
Benzoic acid	Mold and yeast	Bread, pastry, biscuits, wafers, cake creams, soft drinks, fruit juices, table olives, sauces, cocoa products, ketchup, margarine
Propionic acid	Molds and *Bacillus subtilis*	Cookies and cheeses
Acetic acid	Yeast and bacteria	Ketchup, pickles, bread, bakery products

Nutrients

- Foods; It consists of six basic nutrients: carbohydrates, lipids, proteins, vitamins, minerals and water. Microorganisms also need all or some of these nutrients to grow.
- The nutrients that microorganisms need or can use differ from each other.
- The nutrients that microorganisms can use are usually determined by the enzymes they have.
- Microorganisms, with the enzymes they have, break down the nutrients in large structures in sizes that can be taken into the cell and use the nutrients they take into the cell according to their needs. As a result, it produces various substances called metabolites.
- Carbohydrates are the most important source of energy for microorganisms that grow in food. Small-structured carbohydrates such as glucose can be taken directly into the cell, while large-structured carbohydrates such as starch and cellulose can only be used by those with amylase, which breaks down starch, and cellulase, which breaks down cellulose.

Nutrients

- Proteins in foods are not structures that microorganisms can use directly. These need to be broken down into amino acid or small peptide molecules that make up proteins.
  - Microorganisms with protease and peptidase enzymes use proteins by breaking them down into amino acids.
- Microorganisms that have enzymes to break down proteins are called **proteolytic microorganisms**.
- Fats (lipids) can be broken down and used by microorganisms with lipase enzyme. Microorganisms that can use fats by breaking down are called **lipolytic microorganisms**.
- Especially fresh and unprocessed foods are rich in vitamins and easily meet the needs of microorganisms. In addition, many microorganisms produce the vitamins they need themselves.
- Microorganisms decompose, use and multiply the nutrients in food, which means that the food is microbiologically spoiled.

Nutrients

- Microorganisms in terms of minimum nutrient requirement;
  Mold > Yeast > Gram (-) bacteria > Gram (+) bacteria

- Generally, aerobic microorganisms are more lipolytic. Lipolytics are also proteolytic.
- In general, Gram (+) bacteria are microorganisms with low synthesizing power.
- Gram (-) bacteria and molds can synthesize most of their nutritional needs.
- Reproductive factors: These are substances that some microorganisms need for development but cannot synthesize, so they must be taken ready-made from outside.

Protective Biological Structures

- The peel of fruits and vegetables, waxy layers, hard shells of nuts are natural barriers for microorganisms.
- Damage to these protective structures increases microorganism entry and damage.
- If the fruits are picked by removing the stem, they are exposed to stem end rot.
- Protective structures can be damaged by insects as well as damage done at harvest.

Nutrients

- The microflora of foods is mixed and they affect each other.
- Some microorganisms prevent the development of others or kill them with the metabolites they produce.
- This phenomenon is called antagonism. The most common is lactic antagonism. LAB affects other microorganisms with substances such as lactic acid, other organic acids, diacetyl, H_2O_2 they produce.
  - Antibiotics: Bacillus subtilis, subtilin, Streptomyces natalensis, natamycin
  - Bacteriocins: They are antibiotic-like substances of protein nature produced by bacteria. Nisin produced by Lactococcus lactis.
  - Other inhibitors: Propionic acid produced by propionic acid bacteria and ethyl alcohol produced by yeast.

Structure of Foods

- The shells of some foods and some layers that make up their shells prevent microorganisms from reaching the nutrients in the food.
- Waxy cuticle layer in the shell of fruits and eggs, hard and tight fibrous shell structure of oilseeds such as hazelnut and walnut can be given as examples.
- In addition, the skin, scales of fish and the mucus layer on it provide protection against microorganisms.
- Injury or cracking of the shells creates a suitable way for microorganisms to reach the nutrients and causes them to develop and spoil the food.

Factors Effecting Microbial Growth in Foods

External factors (Environmental factors);

a. Relative humidity,
b. Composition and density of gases in the environment,
c. Ambient temperature and time
d. Radiation

- The characteristics of the place where the food is located or stored form the external factors. External factors have an important place in the development of microorganisms and can affect internal factors such as Aw and O/R over time and cause changes in the physical properties of food.

Temperature and Time

- One of the most important requirements of microorganisms for their growth is appropriate temperature.
- While microorganisms can die at temperatures above the temperature range in which they can grow, at temperatures below the growth range, they only stop multiplying and continue their existence.
  - It is not possible to kill microorganisms with low temperature.
- Microorganisms are classified according to the temperature ranges in which they grow are divided into three basic classes; as
  - **Psychrophilic** (cold lovers),
  - **Mesophilic** (warm lovers) and
  - **Thermophilic** (hot lovers)

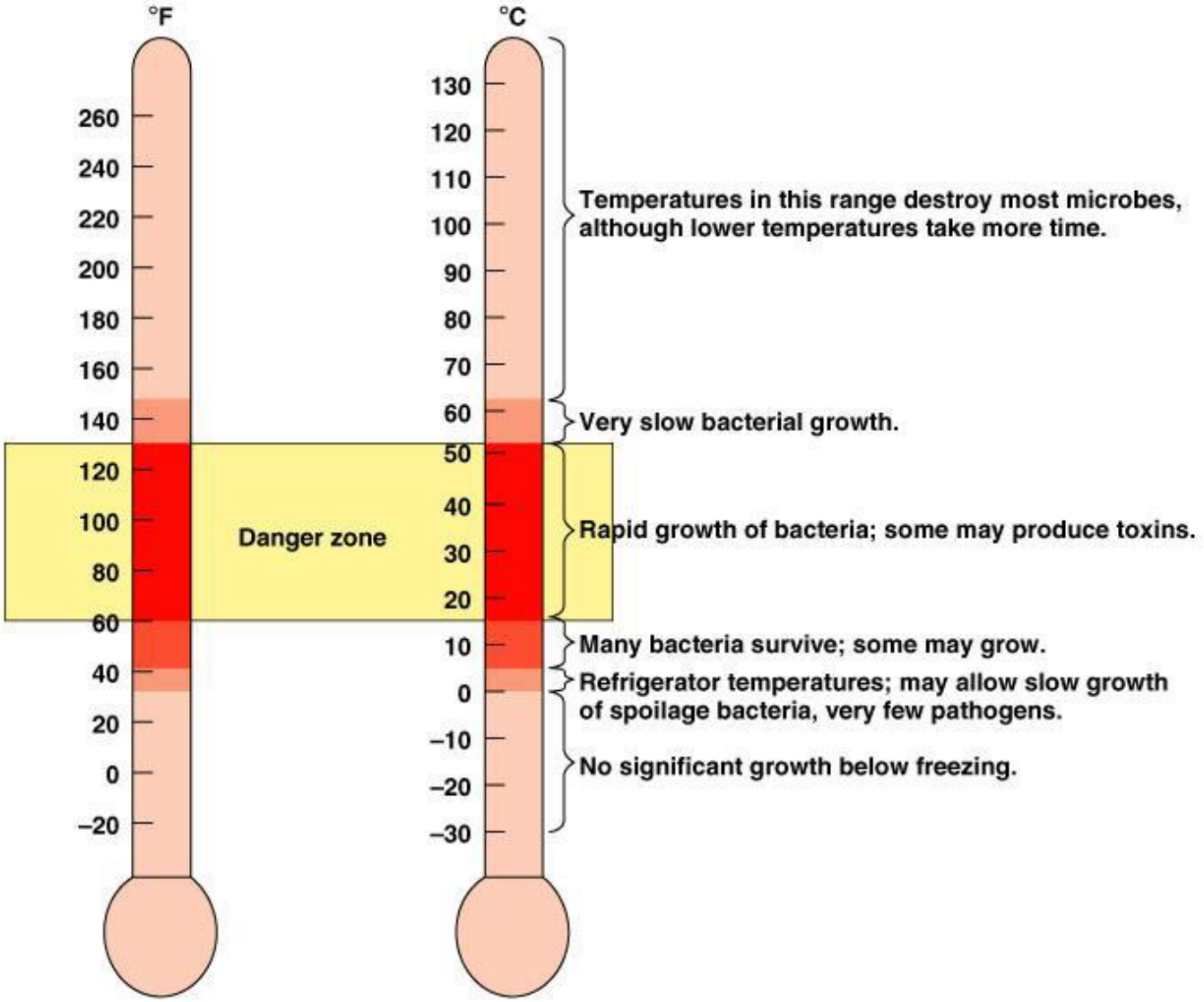
Microorganism Grup	Optimum Growth Temperature (°C)		
	Minimum	Optimum	Maximum
Psychrophilic	0	15	20
Mesophilic	5 - 15	25 - 45	45 - 50
Thermophilic	35- 45	45 - 65	60 - 90

Temperature and Time

- Considering that the majority of pathogenic microorganisms that cause food spoilage and poisoning are mesophilic, storage temperatures below 5 °C and above 65 °C are considered safe.
- In the selection of storage temperature, it should be ensured that the quality characteristics of the food are not adversely affected.
- The storage temperature of foods differs from each other.

Foods	Storage Temperature (°C)	Foods	Storage Temperature (°C)
Pumpkin	+10	Fresh meat	0 - (+1)
Banana	+15	Fish	-1 - (+2)
Potato	+10	Cheese	+1 - (+4)
Beans	+4	Milk powder	+7 - (+21)
Vegetable oil	+21	Butter	+4

Temperature and Time



Relative Humidity

- The relative humidity level of food stores is important in terms of microorganisms that can develop on the surface of the food and the water activity of the food.
- Water molecules always want to move from the environment where they are abundant to the environment where they are less. Therefore, if the humidity of the food is less than the humidity of the surrounding air, the moisture in the air will begin to pass into the food. As the moisture content of the food increases, the amount of Aw will also increase, and microbial growth will be seen in foods with high Aw values. As a result of microbial growth on the food, the food will spoil.
- If the humidity of the food is higher than the humidity of the surrounding air, the moisture in the food will begin to pass into the air. This causes undesirable bruising, shinkage and softening, especially in fruits and vegetables.
- In order not to spoil the food microbiologically and to prevent loss of quality during storage, it must be stored in an environment suitable for the moisture content it contains.

Relative Humidity

Storage Moisture Values of Some Foods and Amount of Water Contained

Food	Storage Relative Humidity (%)	Moisture content of food (%)	Food	Storage Relative Humidity (%)	Moisture content of food (%)
Fresh meat	88-92	62-77	Cucumber	90-95	96
Fresh chicken meat	90-95	74	Lettuce	95	95
Fish	90-95	60-80	Mandarin	85-90	87
Cheese	65-70	30-60	Garlic (dry)	65-70	61
Candies	50	6-10	Dried fruit	50-60	14-26

Gases

- Gases have serious effects on microorganism growth. Especially the gases used in the warehouse environment and in the packaging during storage are directly effective for the microorganisms that develop on the food surface.
- While aerobic microorganisms need oxygen to grow, oxygen has a toxic effect on anaerobic microorganisms. There are also microaerophilic bacteria, molds and yeasts that can grow in environments with very little oxygen.
- There are also microaerophilic bacteria, molds and yeasts that can grow in environments with very little oxygen.
- Carbon dioxide (CO_2), which is present at the rate of 0.003% in the air, adversely affects the development of aerobic microorganisms when it is present in high proportions in the environment. Deterioration is delayed, especially in the storage of vegetables and fruits with carbon dioxide gas.

Gases

- Normal air atmosphere contains certain proportions of O_2, CO_2, N and some other gases. The proportions of gases in the environment affect the growth of microorganism.
- The storage conditions created by adjusting the O_2 and CO_2 ratios in the storage of foods are called "*controlled atmosphere*", and the adjustment of the O_2, CO_2, N ratios in any package is called "*modified atmosphere*".
- In general, Gram (-) bacteria are more sensitive to CO_2 than Gram (+).
- Ozone gas (O_3) is widely used in the disinfection of drinking water.

Oxygen Requirement

$2H_2O_2 \longrightarrow 2\,H_2O + O_2$ (Catalase)

$2O_2^- + 2H_2 \longrightarrow H_2O_2 + O_2$ (Superoxide dismutase)

- **Obligative aerobic**: Oxygen required
  - *Bacillus subtilis,*
  - *Mycobacterium tuberculosis,*
  - *Bacillus anthracis,*
  - *Corynebacterium diphtheriae*

- **Microaerofilic bacteria:** Grow at %1-4 O_2 veya %5-10 CO_2. They need oxygen during their metabolism, but cannot survive at atmospheric density (21%)
  - *Brucella abortus*
  - *Campylobacter jejuni*

Facultative anaerobic bacteria

- Aerobic respiration in the presence of oxygen
- They use fermentation or anaerobic respiration mechanisms in the absence of oxygen.
- Facultative anaerobes use it if it exists, reproduction continues in its absence.
  - *Salmonella,*
  - *Shigella,*
  - *Streptococcus,*
  - *Escherichia coli*
  - *Klebsiella,*
  - *Proteus*

Anaerobic bacteria

- They cannot use oxygen and die in its presence.
- Their catalase and superoxide dismutase enzymes are absent.
  - Obligative anaerobes
    - *Clostridium,*
    - *Fusobacterium,*
    - *Bacteriodes,*
    - *Peptococcus,*
    - *Peptostreptococcus*

Gases

- While the food is being packaged, the air in the package can be vacuumed, **vacuum packaging** or **modified atmosphere packaging (MAP)** can be made by filling the mixture of suitable gases into the package.
- In this way, the development of microorganisms is slowed down and the shelf life of the food is extended. For modified atmosphere packaging, nitrogen (N_2), carbon dioxide (CO_2), carbon monoxide (CO) and oxygen (O_2) gases that are suitable for the characteristics of the food are used.

Radiation (Ionization Radiation)

- Microorganisms are affected by rays of certain wavelengths. In addition to gamma and beta rays, which are called ionizing radiation, ultraviolet light is used in the sterilization and disinfection of foods.
- Gamma and beta rays can affect the inside of the food, but cannot create a significant radioactive residue. In addition, since they cannot increase the temperature of the food too much, the irradiation process with gamma and beta rays is called **cold sterilization**.
- Although ultraviolet rays come to our world from the sun, they can also be created with special lamps. It is used for inactivation of microorganisms on surfaces as it cannot penetrate into the substances.
- Foods subjected to irradiation have the **radura** symbol on their packaging..

Radiation (Ionization Radiation)

The Ministry of Agriculture and Forestry has determined the necessary conditions for food irradiation with the Food Irradiation Regulation as follows:

1. Food irradiation process; It cannot replace good production, good agricultural or good hygiene practices and is only applied when:
    a. If there is a technological need,
    b. If it does not pose a health hazard and is carried out under recommended conditions,
    c. If consumer interests are appropriate

2. The food irradiation process is used only for the following purposes:
    a. Prevention of foodborne diseases by destroying pathogenic microorganisms,
    b. Preventing spoilage of foods by destroying microorganisms that cause spoilage and preventing or delaying decay,
    c. Reducing food losses that may occur with sprouting, germination and ripening,
    d. Removal of organisms that harm plants or plant products from food.

The Concept of Hurdle (Combination of Factors)

- The concept of creating a hurdle (hurdle concept) refers to the regulation of many factors together in the control of spoilage in foods or the development of pathogenic microorganisms.
  - Obstacle technology
  - Combined protection
  - Combined techniques
- The terms are also used to describe the same concept.