FE 305 FOOD MICROBIOLOGY
Food Preservatives and Fermentation

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Food Preservatives

- Different heat treatments, freezing, drying and irradiation are used in order to prevent food spoilage, ensure food safety and control microorganisms in food, and in this way, vegetative forms and spores of microorganisms are killed.
- However, since there are significant losses in the nutritional value of the food with heat treatments and the stability of the food must be preserved for a long time after opening, the use of protective additives becomes mandatory.
- Preservatives are defined as chemical substances that extend the shelf life of food by protecting the food against spoilage caused by microorganisms (bacteria, yeast, mold).
  - For example, nitrite, sodium benzoate, sorbate, parabens.
  - However, although salt, sugar and vinegar have been used to prevent microbial spoilage since ancient times, these substances are not considered additives.

Food Preservatives

- Substances used as preservatives are also called antimicrobial agents.
- Antimicrobial substances are substances that stop the growth or kill the microorganisms that are in the food or that will be contaminated.
- Preservatives, also referred to as antimicrobials, act on certain microorganisms.
- In addition to nutrients, pH, water activity, humidity, temperature, inhibitory substances and environmental factors are also important in terms of microbial growth.

Food Preservatives

- The amount of preservatives to be used and in which foods are determined by law.
- Many of the preservatives are mixed into foods. Some preservatives are only allowed on the surface, as in cheese and citrus.
- The expiry date of the food is determined by determining how many days or months the preservatives are effective.
- Unconscious use can affect human health in the first degree.
- For this reason, before using antimicrobial substances, food manufacturers must obtain information from the producers producing these substances and related laws.

Important preservatives used in the food industry

- Sulfur dioxide and various sulfites
- Nitrite and nitrate compounds
- sorbic acid
- Propionic acid
- Acetic acid
- Benzoic acid and its salts
- Natamycin with antimycotic effect
- Some glyceryl esters
- Some antibiotics, such as nisin.
- Antimicrobials are numbered in the range of E and 200-290 in the INS system.

Organic acids

- Organic acids and their esters are widely found in nature, especially in fruits.
- It also extends the shelf life of the product with its retarding and inhibitory effect on proteolytic deterioration.
- It has been determined that acetic acid is effective against yeast and bacteria.
- Sodium Benzoate salt has long been used as a preservative in foods.
  - It is stated to be active against yeast and bacteria and less active against molds.
- Propionic acid is especially effective against mold and rop disease in breads.

Sorbic Acid

- Sorbic acid, sodium (Na) and potassium (K) salts are chemical substances that are effective against mold and yeast.
- In the Food Industry used in;
  - various cheese and cheese products,
  - ketchup and mayonnaise,
  - Pickles, pickled and table olives,
  - dried fruits,
  - fruit juices,
  - Meat and meat products,
  - Bakery products
  - jam, bread and syrups.
- Although sorbic acid is an alternative preservative as effective as nitrite and does not cause carcinogenic problems, it is not used in meat products because it does not provide color and flavor formation..

Benzoic Acid

- Benzoic acid and its salts are effective against yeast and molds.
- Benzoic acid can cause asthma and allergies in humans.
- The sodium salt of benzoic acid is generally used in foods.
- It affects the taste of the food to which it is added. It is used in;
  - sauce, ketchup, mayonnaise,
  - Pickles, olive paste, table olives
  - Margarine, marmalade juice and preserves,
  - carbonated drinks,
  - egg products.

Sulfur dioxide and sulfites

- As a preservative, either sulfur dioxide gas directly or sulfur salts (sulfides) that produce sulfur dioxide gas when decomposed are used.
- Sulfur dioxide is especially effective against microorganisms in acidic foods.
- In addition to its antimicrobial effect, sulfur dioxide prevents browning in foods, so dried fruits such as apricots are also used.
- Adverse effects such as headache, nausea, breathing difficulties, itching, redness and allergies may be seen in patients with sulfide-sensitive asthma.
- Foods containing sulphites, which are consumed in the amount permitted by law, do not affect the majority of consumers, except for asthma patients.
- Although sulphites are in different forms, the most effective form is sulfurous acid. Bacteria are more affected by sulfur dioxide than yeast and molds.

Sulfur dioxide and sulfites

- It is frequently used in the meat industry in alcoholic beverages, drying fruits and vegetables, frozen and pickled fruit juices.
- Sulfur dioxide is also used against mold in cheese ripening rooms and warehouses.
  - Sulfurization process is one of the basic processes applied especially in getting rid of seedless grapes and apricots.
  - Dried apricots get their bright yellow color only in this way.
  - Sulfur dioxide and sulfites such as fish, meat and seafood, dried tomatoes, dehydrated vegetables, dried fruits, vinegar, sausage, mustard, liquid pectin, potato products, glucose syrup, marmalades, fruit and vegetable purees.
  - The use of sulfites is not allowed in foods that are an important source of thiamine (vitamin B1), such as meat, to avoid vitamin loss.
- It is used as a preservative in disinfecting food tools and equipment and bottles.
- The ADI value of sulfur dioxide is 0.7 mg/kg. In other words, a normal person weighing 50 kg can consume a maximum of 35 mg of sulfur dioxide product per day.

Nitrites ve Nitrates

- Sodium and potassium salts of nitrates and nitrites are curing agents used in meat and fish products to give characteristic flavor and to maintain microbial balance.
- If preservatives are not used in meat products, the development of *Clostridium botulinum* bacteria, which causes botulism, cannot be prevented and food poisoning may occur.
- It is a preservative that is used in cured meat products and some cheeses and has an antimicrobial effect.
- In addition to the antimicrobial effect, nitrates and nitrites form the typical color in heat-treated meat products such as salami, sausages and fermented meat products such as sausage.
- Nitrate is also used in canning cheese and fish.
- Although nitrite is a toxic substance, it is used as an additive in meat products because there is no alternative.

Nitrites ve Nitrates

- Excessive consumption of nitrate and nitrite-containing foods can adversely affect human health.
- Because nitrate and nitrite can turn into substances called "Nitrosamine", which have carcinogenic effects in the body.
- Vitamins C and E can prevent the formation of nitrosamines.
- Therefore, it is recommended to drink a fruit juice containing vitamin C, such as orange juice, for the consumption of foods containing nitrates and nitrites.
- A large portion of dietary nitrate intake comes from vegetables.
- Nitrate can be found in high amounts in vegetables such as cabbage, broccoli, cauliflower, carrots, celery, lettuce, radishes, beets and spinach, with 86% of daily nitrate intake coming from vegetables.
- Since the fertilization method is applied incorrectly in many places, nitrates are also found in green leafy vegetables and salads.

Nisin

- Nisin is a preservative with an antibiotic effect.
- It can be produced from the bacterium *Lactococcus lactis* by microbial synthesis.
- Studies have shown that it has no toxic effects.
- It can be used in milk and dairy products, canned food, soup, melted cheese, meat and meat products.
- It is allowed to be used in matured and processed cheese, semolina and sour cream in Türkiye.

Natamycin

- Natamycin is an antibiotic effective against yeasts and molds.
- It was first obtained from the microorganism *Streptomyces natalensis* in 1955.
- It is used on the surfaces of hard and semi-hard cheeses and sausages.
- Animal studies have not found any toxic effects of natamycin.
- Natamycin is allowed to be present up to 5 mm from the surface of the products into the interior.
- It has been reported that the maximum amount of natamycin allowed on the cheese surface can be 1 mg/dm^2.

Dimetyl dicarbonate (DMDC)

- This colorless, fruit-smelling liquid substance is effective on fungi (yeast and molds). It can be used as a fungicide in soft drinks and as a sterilant against yeasts in wine.

Sterilant Gases

- Sterilant Gases are additives that protect foods against spoilage.
- Today, Carbon dioxide (E 290), Sulfur dioxide (E 220), ethylene oxide, propylene oxide and ozone gas are gaseous preservatives used in foods.
- According to carbon dioxide, food and microflora (colorless, odorless and non-flammable at normal storage temperatures. Molds are sensitive to carbon dioxide and yeasts are resistant to some extent. Bacteria can show very different reactions according to their oxygen needs.
- Ethylene oxide is a gas that is used effectively as a food preservative.
- Carbon monoxide, at a concentration of 1%, inhibits the growth of psychrophilic microorganisms.
- Ozone is an unstable bluish water-soluble gas. Bacteria are more sensitive to ozone than molds and yeasts. It is especially used for sterilization of water.

Antibiotics

- Antibiotics show antimicrobial effect by inhibiting the enzyme activities of microorganisms or the production of basic building blocks.
  - Tylosin, nystacin, natamycin, subtilin, nisin can be given as examples.
- Other preservatives: Substances such as formic acid, boric acid, lysozyme, which are not included in the above-mentioned groups, are substances used as food preservatives.

Fermentation

- Fermentation is the breakdown of high molecular substances, especially carbohydrates, into smaller substances by microorganisms.
- Fermentation technology is an important evaluation group in agricultural technology.
  - Especially wine, beer, vinegar and spirit technologies, pickles produced by lactic acid fermentation, olive, boza, tarhana etc. products are one of the important topics of fermentation technology.

Fermentation

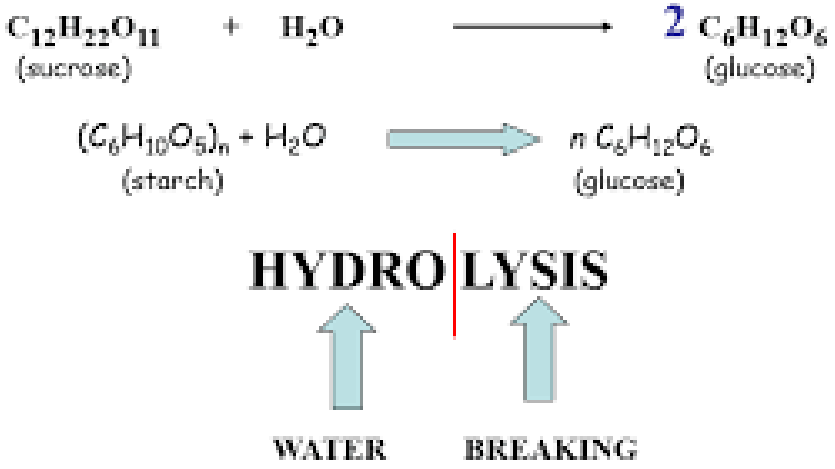
- In fermentation technology, biochemical events form the basis of the process.
- Therefore, it is of great importance to know the composition of the raw material used and the physical, chemical and microbiological properties of the elements in the composition.
- In fermentation technology, carbohydrates in the composition of raw materials are directly involved in the process.
- With the breakdown of carbohydrates, alcohol, acetic acid, lactic acid, etc. compounds such as
- Although nitrogenous substances and mineral substances in the raw material are auxiliary elements in processing, they are especially necessary for the survival of microorganisms.
- Acids, on the other hand, play an important role in the optimum functioning of microorganisms and enzymes by affecting the pH of the environment, and also on taste. Vitamins increase the nutritional value of the product. Flavor (smell, taste and aroma substances) substances, on the other hand, increase the quality of the product.

Raw Materials

- The most important compounds in fermentation technology are carbohydrates.
- In addition to these, aroma substances, vitamins, organic acids are also important.
- They are formed by the fermentation of carbohydrates.
- Fermented carbohydrates are monosaccharides.
- There may not be any monosaccharide to be fermented as a raw material.
- For example, carbohydrates in cereals are in the form of starch. It must first be converted into simple sugars by hydrolysis by acid or enzyme, and then undergo fermentation.
- No polysaccharide undergoes fermentation. Starch is broken down to glucose with the help of enzymes, then it is fermented.

Carbohydrates

- Carbohydrates are organic compounds made up of C, H, and O.
- They are commonly found in plant and animal tissues.
- Besides being a source of energy, they also have separate functions.
- Carbohydrates are aldehydes and ketones of polyhydroxy alcohols chemically containing more than one OH group.
- They are denoted by the general formula $[CH_2O]n$.
  - Here n; is at least 3. There are non-carbohydrate substances that fit this formula, and there are also compounds that are carbohydrates but do not fit this formula.



Objectives of Fermentation

1) Improving Food Security

a) Food preservation

- a cheap and energy efficient
  - means of preserving perishable raw materials.

b) Salvaging (reduce) waste foods

- Fermentation produces food without waste
  - by changing the consistency of the product and making it digestible.
- This increases the range of raw materials available as food.
  - Such as pineapple peel vinegar from fruits, Tempe from fermenting peanut and coconut press-cake.

c) Removal of anti-nutritional factors

- Many foods contain naturally toxins and anti-nutritional compounds.
  - These can be removed or detoxified by the action of microorganisms during fermentation.

Objectives of Fermentation

d) Antimicrobial activities

- Fermentative microorganisms produce different organic compounds and chemicals;
  - low pH, organic acids, bacteriocins, H_2O_2, ethanol, nutrient depletion, low redox potential, etc.

2) Increasing Income and Employment

- The production of fermented food products provides income and employment to millions of people around the world.

3) Health-Benefits

- Improving nutrition, produce vitamins, increase digestibility, reduce lactose intolerance, probiotics, cholesterol, anticancer effects, stimulate immunological system, medical benefits.

4) Improving Cultural and Social Well Being

- Fermentation can improve the flavor and appearance of food.

5) Malo-Lactic Fermentation

- LAB can decarboxylate L-malic acid to produce L-lactate in malo-lactic fermentation of wine.

Importance of Fermentation

- Improvement and/or enrichment of the human diet by the formation of aroma substances.
- Making food stable (Lactic acid, Alcohol, Acetic acid and alkali fermentations).
- Biological enrichment with protein, essential amino acids, essential fatty acids and vitamins.
- Breakdown (detoxification) of anti-nutritive substances during the fermentation process.
- Improving the digestion of food, reducing the preparation time and energy requirement.

Fermentation Types

1. Solid-state fermentations;

- Microorganisms grow on a moist solid food with little free water.
- Inoculate microbial culture onto surface area of solid media.
- Solid-state fermentations are O_2-reduced aerobic process and anaerobic process.
  - Aerobic ferm.: citric acid production by *A. niger*.
  - Anaerobic ferm.: koji fermentations (to produce soy sauce) by *A. orycaeus*;
    - » sucuk fermentations by *Lactobacillus*, *Pediococcus*, *Staphylococcus* and *Micrococcus*.
    - » bread making and processing of cocoa and tempeh.

Fermentation Types

2. Submerged fermentations;

- Microorganisms grow in a liquid medium with mixing the culture.
- Inoculate microbial culture onto liquid medium.
- grow on a moist solid food.
- They are anaerobic process.
- Submerged fermentations may use dissolved substrate,
  - e.g. sugar solution or a solid substrate, suspended in a large amount of water to form slurry.
- Examples are pickling vegetables, yogurt, beer and wine.

Fermentation Microorganisms

- Microorganisms that play a role in fermentation technology are grouped under three main groups in order of importance:
  - Yeast
  - bacteria
  - Molds
- These are known as starter cultures.
- Features that should be found in starter cultures.

1. Hygienic compatibility

   1.1 The starter organism must be non-pathogenic and non-toxic.

   1.2 Starter culture preparations should be hygienically free from infection and compounds.

2. Technologically efficient

   2.1 The culture organism must multiply and dominate over the spontaneous microorganisms.

   2.2 The culture organism must be able to work effectively in the way it is used (nitrate reduction, biological acid degradation, etc.)

   2.3 Starter culture preparations must be technologically free from infection and compounds.

Fermentation Microorganisms

- Microorganisms used in the fermentation industry are the main factors in the success of any fermentation. Because the catalyst of fermentation is microorganism.
- It is therefore natural to seek some process-appropriate traits from a microbial culture.
- These are generally:

  a. The strain must be genetically stable. A strain that continuously and spontaneously produces one or more forms is undesirable.

  b. The strain should be able to easily produce a large number of vegetative cells, spores or other reproductive organs.

  c. The strain should be able to multiply rapidly and vigorously following inoculation.

  d. The strain culture should be clean not only from other microorganisms visible under the microscope, but also from phages.

  e. The Strain in a short time; it should be able to produce the desired product, preferably in 3 or less days.

Fermentation Microorganisms

f. The strain should form a clean product of all toxic substances. In addition, this product should be easily separated from all other materials.

g. The strain should be able to protect itself, if possible, against contamination. This self-protection can be achieved by lowering the pH of the medium, proliferating at high temperatures, and easily creating a desired microbial inhibitor.

h. The strain should be easily maintained for a reasonable period of time.

I. The strain must be able to be mutated by some mutagen or a group of mutagens. A mutation program can be made to obtain high yielding strains.

j. The strain should yield the expected amount of product within a certain fermentation time.

Yeasts

- In fermentation technology, when yeast is mentioned, suitable strains belonging to the *Saccharomyces* genus are understood.
- Wild yeasts, on the other hand, are yeasts that are not used in industry and have undesirable and harmful activities.
- Yeasts contain, on average, 70-75% water and 25-30% dry matter.
- 50-60% of dry matter is protein, 25-35% is carbohydrates, 6-9% is minerals and 4-7% is fat.

Bacteria

- Bacteria are generally harmful microorganisms because they cause disease and spoilage.
- In fermentation technology, bacteria that produce acetic acid and lactic acid are used.
- Those that produce acetic acid are called acetic acid bacteria, and those that produce lactic acid are called lactic acid bacteria.

Molds

- Although molds are generally harmful microorganisms, there are some that are used for some purposes in the industry.
- For example, *Aspergillus niger* is used as a malt substitute to break down starch in the production of citric acid from sugar and in the production of spirits from cereals because it is rich in diastase enzymes.
- However, *Aspergillus oryzae* is also used in making sake, a Far Eastern drink.