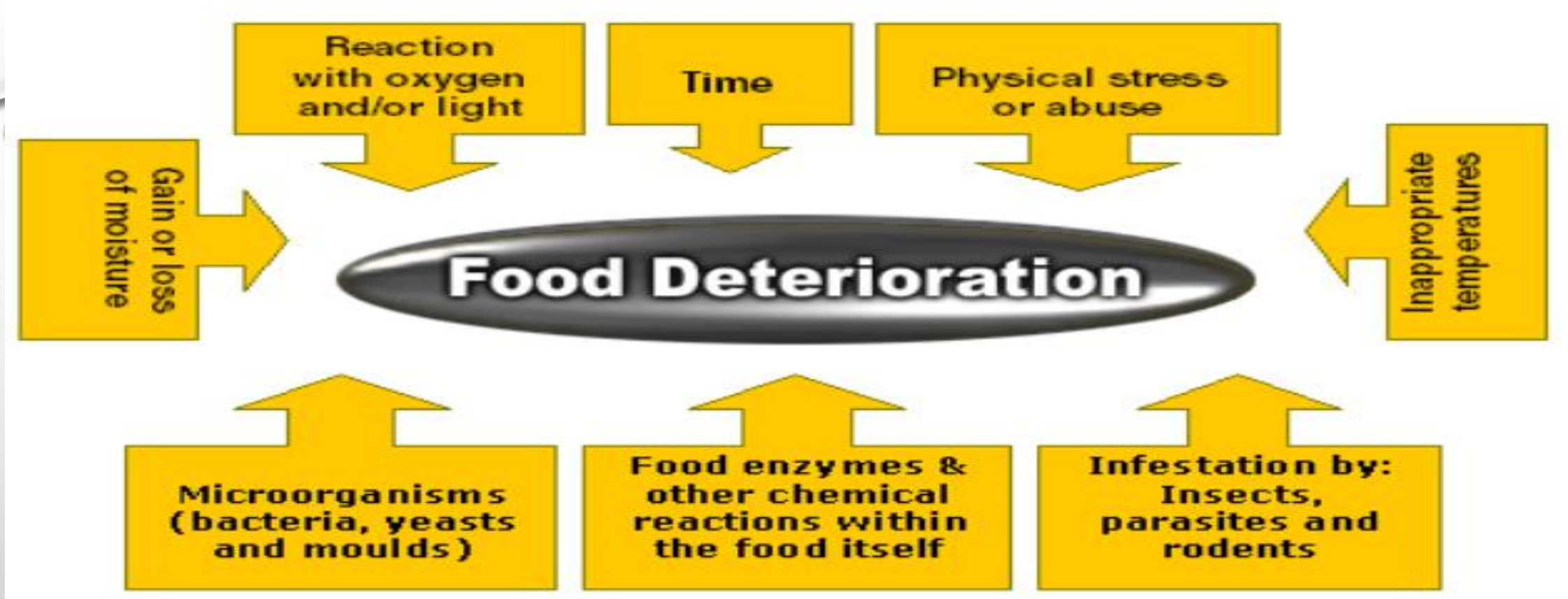
COURSE TITLE: FOOD AND INDUSTRIAL MICROBIOLOGY
COURSE NO. - DTM-321: CREDIT HRS-3 (2+1)

FOOD PRESERVATION
CHEMICAL METHODS

RAKESH KUMAR
ASSOCIATE PROFESSOR (DAIRY MICROBIOLOGY)
FACULTY OF DAIRY TECHNOLOGY
S.G.I.D.T., BVC CAMPUS,
P.O.- BVC, DIST.-PATNA-800014


Reaction with oxygen and/or light
Time
Physical stress or abuse
Gain or loss of moisture
Food Deterioration
Inappropriate temperatures
Microorganisms (bacteria, yeasts and moulds)
Food enzymes & other chemical reactions within the food itself
Infestation by: Insects, parasites and rodents


Chemical method
Preservation by vinegar
Preservation by sugar
Preservation by oil
Sun drying
Salting
Pasteurisation
Boiling & freezing

According to FSSAI rules → class I and class II preservatives

Class I preservatives

a. Common salt
b. Sugar
c. Dextrose
d. Glucose
e. Spices
f. Vinegar or acetic acid
g. Honey
h. Edible vegetable oil

Class II preservatives

a. Benzoic acid including salts
b. Sulphurous acid including salts
c. [Nitrates of] nitrites of sodium or potassium
d. Sorbic acid including its sodium, potassium and calcium salts
e. Niacin
f. Propionic acid including salts
g. Methyl or propyl para-hydroxy benzoate
h. Sodium diacetate
i. Sodium, potassium and calcium salts of lactic acid

Addition of class I preservatives in any food is not restricted. Use of class II preservatives is restricted. They shall be added to only specified product and at a concentration not exceeding the proportion specified for the product. Use of more than one class II preservative is prohibited. No person shall use in or upon a food more than one class II preservative

Chemical Preservatives

- ✓ The purpose of using a chemical agent as a preservative is to retard food spoilage caused by microorganisms.
- ✓ As per one estimate of WHO, it was declared that 20% of the world's food is lost by different type of spoilages.
- ✓ Partial prevention of this spoilage can be achieved through the use of refrigeration, drying, freezing and fermentation.
- ✓ The use of chemical additives or preservatives will prolong the shelf life of the food even further.

Mode of action of Chemical preservatives

Interfere with the cell membranes of microorganisms, their enzyme activity or then genetic mechanisms.

Preservatives may also serve as

- ➢ antioxidants,
- ➢ stabilizers,
- ➢ firming agents,
- ➢ moisture retainers.

At what stage it should be added in food

Chemicals that function to preserve the food are generally added after the food has been processed and before it is packaged.

Chemical Preservatives == Certain preservatives have been used either accidentally or intentionally for centuries includes sodium chloride (common salt), sugar, acids, alcohols and components of smoke. In addition to preserving our food, these compounds also contribute to the quality and specificity of the products and these are applied through processing procedures such as salting, curing, fermentation and smoking.

Different chemical preservatives are used, which involves

1. Traditional chemical food preservatives Sugar Salt
2. Acidulants Benzoic acid Sorbic acid Lactic acid
3. Gaseous chemical food preservatives/ leavening agents Sulphur dioxide and sulphites Carbon dioxide
4. Antioxidants Butylated Hydroxy Anisole (BHA) Butylated Hydroxy Toluence (BHT) Propyl Gallate Natural / Synthetic Tocopherols (Vitamin E) Ascorbic Acid (vitamin C) and Lecithin.
5. Colour additives
6. Flavour additives
7. Sweeteners
8. Emulsifiers

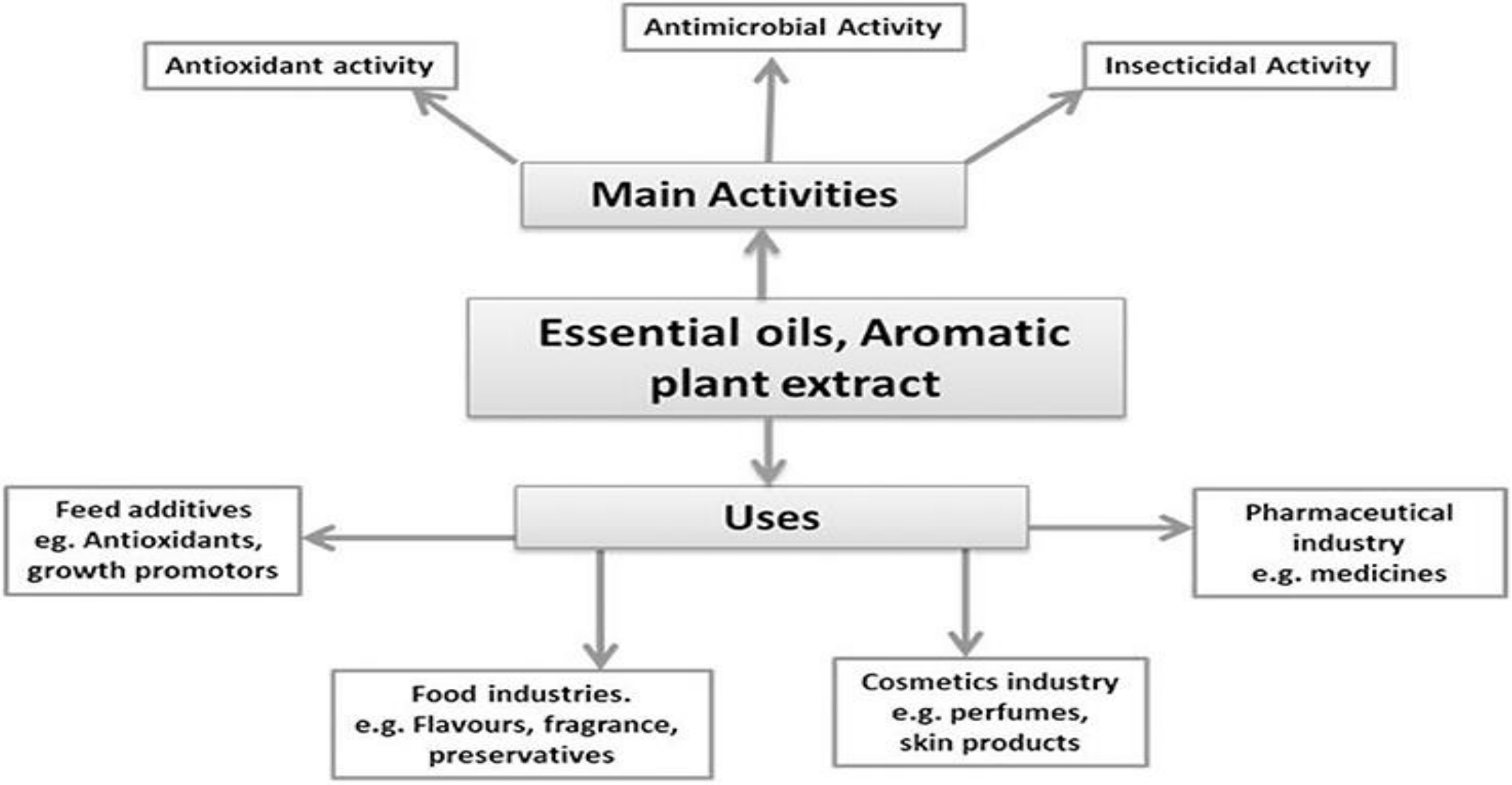
Site of preservative activity in microbial cell

Cell Wall	Cytoplasmic membrane	Cytoplasm
Phenols	2-Phenoxyethanol	2-Phenoxyethanol and other organic alcohols
Aryl and alkyl acids	Parabens	Aryl and alkyl acids
Organo mercurials	Organo mercurials	Halogenated preservatives
EDTA (edetic acid)	EDTA	
Chlorhexidine, cetrimide	Chlorhexidine, hexachlorophene	Chlorhexidine (high concentrations)
Glutaraldehyde	Formaldehyde donators e.g. bronopol, imidurea	Formaldehyde donators e.g. bronopol, imidurea
Anionic surfactants	Benzalkonium chloride (BKC)	

Vegetable oil and Aromatic Plant extract

Vegetable oil consists of monoglycerides, Diglycerides and triglycerides.

Mechanism of action: In pickles it prevents the entry of micro organisms by forming an impervious layer. It also prevents the entry of oxygen and creating anaerobic condition.

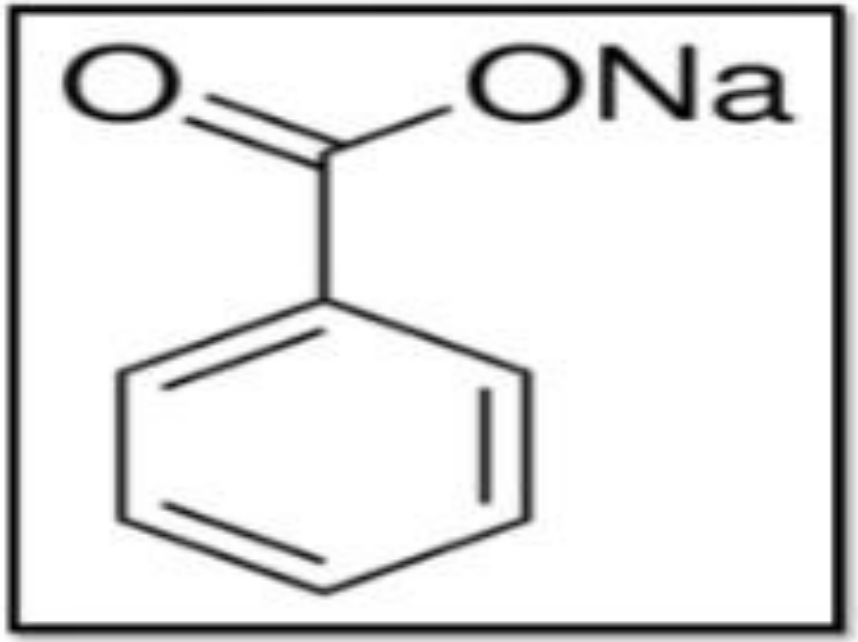



Benzoic acid

Benzoic acid in the form of its sodium salt constitutes one of the most common chemical food preservative and Sodium benzoate is a common preservative in acid or acidified foods such as fruit juices, syrups, jams and jellies, pickles, preserves, fruit cocktails, etc. Yeasts are inhibited by benzoate to a greater extent than are molds and bacteria. Greatest activity at low pH and become ineffective as the pH increases towards neutral values. The **mechanism** starts with the absorption of **benzoic acid** into the cell. If the intracellular pH changes to 5 or lower, the anaerobic fermentation of glucose through phospho-fructokinase is decreased by 95%. It is most effective in low pH foods (fish sauce, tomatoes sauce, soft drinks etc). It also affect microorganisms by inhibiting cellular uptake of substrate molecules. Generally, the stage of endospore germination is most sensitive to benzoates. Maximum permissible level in foods is 0.1%.

It is used in the form of sodium benzoates in the preservation of fruit juices and squashes.

Mode of Action: It inhibits the growth of micro organisms by inhibition of action of enzymes that control the acetic acid metabolism and oxidative phosphorylation. it inhibit the growth of yeasts and moulds.



Advantages:

- It is used in colored juices
- Most active against yeast and moulds

Sulphur dioxide and sulphites

SO2 and sulphites may be added to such foods as dried fruits, fruit pulp, juices and molasses. They are used to conserve colour, act as antioxidants and control microbial growth. Sulphur dioxide and its various sulphites dissolve in water and at low pH levels yield sulphurous acid, bisulphite and sulphite ions. The various sulphite salts contain 50-68% active sulphur dioxide. A pH dependent equilibrium is formed in water and the proportion of SO2 ions increases with decreasing pH values. At pH values less than 4.0 the antimicrobial activity reaches to its maximum.

In the past cut fruits and vegetables in salads bars were sprayed or dipped in a solution of sulphites. Sulphur also inhibits enzymatic browning and keeps plant tissue fresh for longer period. But this practice has been banned by the FDA because there were over 100 reported cases of adverse reactions including death. It is also reported that approximately 5-11% of asthmatics are sensitive to sulphates and experience symptoms such as headaches, nausea, abdominal pains and dizziness.

Sulphur Dioxide

SO_2 - Aerobic microorganisms are more sensitive

- Yeasts are less sensitive than molds, acetic acid bacteria and lactic acid bacteria.
- Molds (Botrytis sp) on grapes are controlled by periodic gassing with SO_2.
- Bisulphites are used to destroy aflatoxins in foods and used at ppm level.

Mechanism of action:

Antimicrobial activity is because of the formation of undissociated sulphurous acid or gaseous sulphur dioxide. Due to its strong reducing power it lowers the oxygen tension below the level required by aerobic organisms and by the direct action on certain enzymes it inhibits microorganisms. Metabisulphites also affects vegetative cells during endospore germination.

Advantages:

- It has better preserving action against bacterial fermentation
- It helps in retain the colour of the beverage for long time
- It has strong effect in retarding oxidation
- Preventing discoloration and loss of flavor in products
- It is highly soluble in squashes and juices ensures better preservation

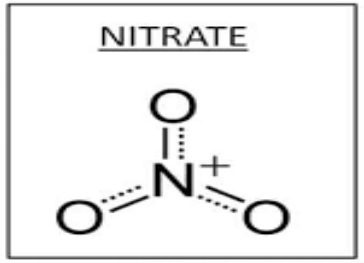
Disadvantages:

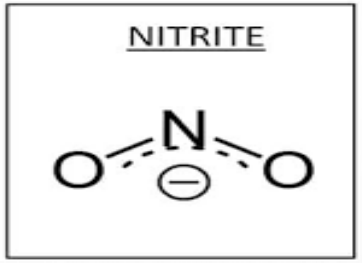
- It cant be used in naturally colored juices
- It should not used in juices that are stored in tin containers

Permissible limit of preservatives in food products

Clip slide

Sl. no	Food product	preservative	ppm
1	Fruit pulp or juice for conservation in to jam or jelly and other products		
	Cherries Berries Other fruits	Sulphar dioxide --do- --do-	3000 2000 1000
2	Fruit juice concentrate	--do-	1500
3	Dried fruits Temperate fruits Raisins	--do- --do-	2000 750
4	Squashes crushes syrup cordial and juices	Sulphar dioxide Or benzoic acid	350 600
5	Jam, marmalade, preserve, fruit jelly	Sulphar dioxide Or benzoic acid	40 200
6	Fruit and fruit pulp	Sulphar dioxide	350
7	RTS sweet	Sulphar dioxide Or benzoic acid	70 120
8	Pickles and chutneys	Sulphar dioxide Or benzoic acid	250 100
9	Tomato and other sauces	benzoic acid	750





Nitrates and Nitrites –

- Sodium nitrite and sodium nitrate - used in meat curing, help to stabilize pink meat colour, flavor, inhibit spoilage and food poisoning organisms and contribute to flavour development. They function as preservatives, helping to prevent the growth of harmful bacteria.
- Have been used as preservatives for meats for centuries especially in hams, bacon, bologna hotdogs and sausages.
- Nitrites prevent the growth *Clostridium botulinum* responsible for secreting a deadly toxin these micro organisms grow in anaerobic condition readily found in the interior of ham or in meat that has been vacuum package.
- Nitrite is highly reactive and can serve as both oxidizing and reducing agent. Nitrite in acid environment ionizes to nitrous acid which further decomposes to nitric oxide. This nitric oxide reacts with myoglobin under reduced condition to produce desired red pigment, nitroso myoglobin. Nitrites are effective against several food poisoning (Clostridium sp) and spoilage microorganisms.

The inhibitory or antimicrobial effect results from heat processing or smoking of meat and fish products containing nitrite. Use of nitrite – for preventing food poising due to Clostridium rather than color and flavor development. Using at levels of 120 ppm causes antimicrobial effect, and ppm helps in fixing color and flavor development

Made of action: Antimicrobial effect of nitrite is because of the inhibition of vegetative cell growth and growth of spores that survive heat processing/smoking during post processing storage. The antimicrobial effect of nitrite is due to inhibition iron-sulphur enzymes present in the microorganism. Same effect of nitrite on botulism inhibition observed due to its effect on iron-sulphur enzymes thus preventing synthesis of ATP from pyruvate.

Sorbic acid and sorbates

Sorbic acid --- It is used in food preservation as salt of calcium, sodium and potassium. Sorbic acid and its salts are practically tasteless and odorless in foods. The maximum allowable level in foods is 0.20% and Sorbates are most effective in acid foods (pH below 6) than neutral foods. Not effective in pH > 6.5. The undissociated form is responsible for antimicrobial activity. It is considered non toxic and easily metabolized. Among other common food preservatives the WHO has set the highest acceptable daily intake (25 mg/kg body weight) for sorbic acid. Sorbates are used for mold and yeast inhibition in a variety of foods including fruits and vegetables, fruit juices, pickles, syrups, jellies, jams, preserves, high moisture dehydrated fruits, etc. It is highly effective against *Staph aureus*, *Salmonella*, *Coliforms*, Psychrophilic spoilage bacteria, *Vibrio parahaemolyticus* and others.

Potassium sorbate, a white, fluffy powder, is very soluble in water (over 50%) and when added to foods acidic in nature, it is hydrolyzed to the acid form. Sodium and calcium sorbates also have preservative activities but their application is limited compared to that for the potassium salt, which is employed because of its stability, general ease of preparation and water solubility.

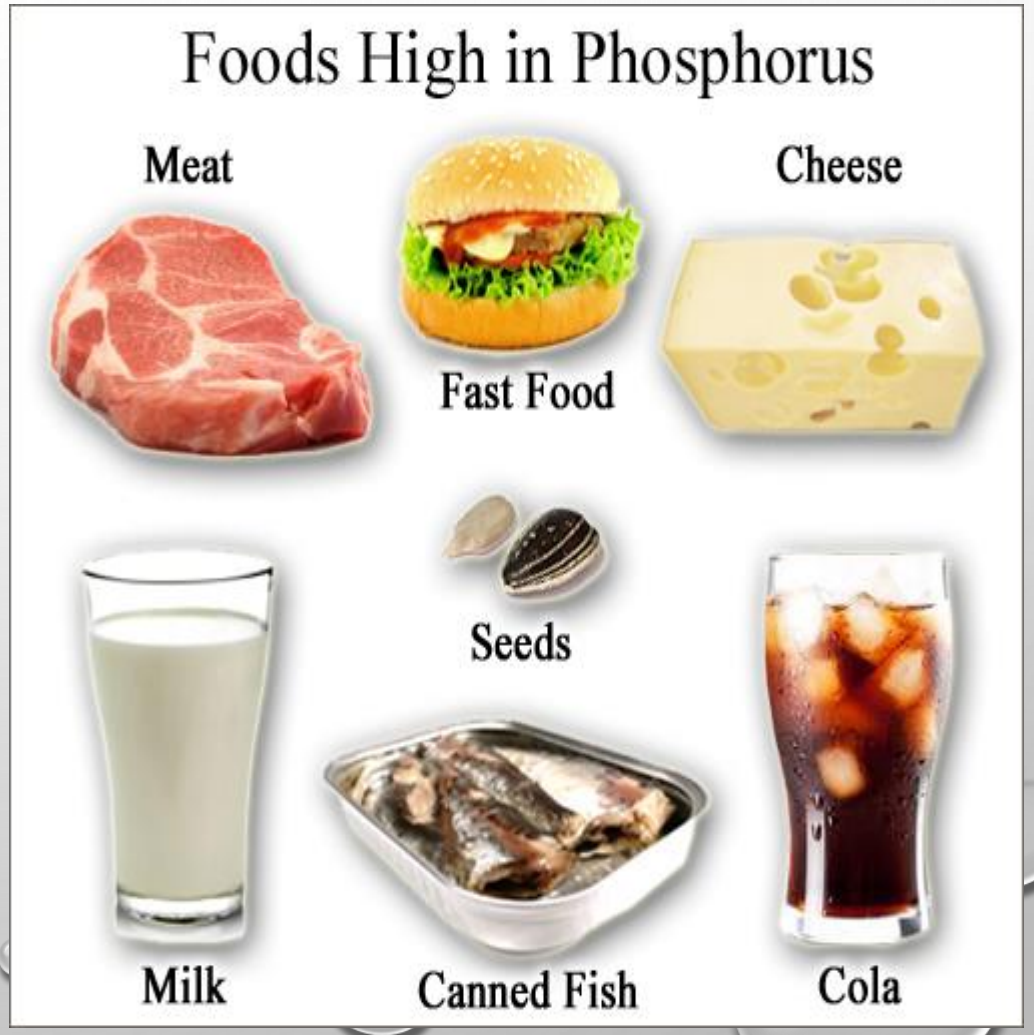
Sorbic acid and sorbates effect on microorganisms

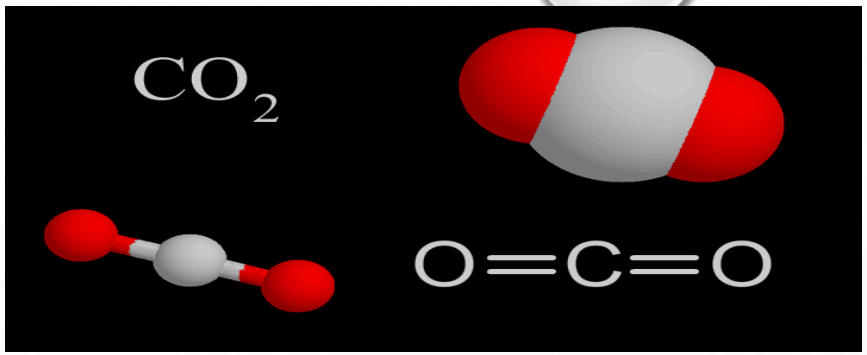
- ✓ Inhibition of molds is due to the inhibition of dehydrogenase activity.
- ✓ Catalase positive cocci are more sensitive than catalase negative forms
- ✓ Aerobes are more sensitive than anaerobes.
- ✓ It also prevent growth of vegetable cells that are germinating from endospores.

Antimicrobial activity is due to undissociated form. These chemicals affect proton motive force of bacterial cells. Being lipophilic in nature it acts on cytoplasmic membrane of microorganisms and separate proton (H+) and hydroxyl ions. The H+ ions move outside the cell and cause acidic pH while the OH- ions increase pH inside the cell near neutrality. At this pH sorbates inside the cell dissociate and cause lowering of intracellular pH. These results in weakening of transmembrane gradient required for transport for amino acids to inside cell, thus adversely affecting membrane transport and causing subsequent cell death.

Phosphates

These salts are commonly added to certain processed meats to increase their water holding capacity. They also contribute to flavour and antioxidative activity. Food grade phosphates range from one phosphate (e.g. trisodium phosphate) to at least 13 (sodium polyphosphate). They possess anti botulinal activity, especially when combined with nitrites. Combination of 140 ppm $NaNO_2$, 0.26% potassium sorbate, and 0.14% sodium acid pyrophosphate (SAPP) delay *C. botulinum* neurotoxin production.





Carbon dioxide

Carbon dioxide is used as a solid (dry ice) in many countries as a means of low temperature storage and transportation of food products. Beside keeping the temperature low, as it sublimes, the gaseous CO2 inhibits growth of psychrotrophic micro-organisms and prevents spoilage of the food (fruits and vegetables, etc.). Carbon dioxide can also be used as a direct additive in the storage of fruits and vegetables.

Carbon dioxide

It is used in the controlled/ modified environment storage of fruit and vegetables.

The correct combination of O2 and CO2 delays respiration and ripening as well as retarding mold and yeast growth resulted in an extended storage of the products for transportation and for consumption during the off-season. The amount of CO2 (5-10%) required is dependent upon the factors such as nature of product, variety, climate and extent of storage.

Colour Additives

Colour Additives

The acceptance of food products is determined largely by its appearance, consumers become accustomed to standardized colors in familiar foods and base their purchasing habits / decisions or past experiences. In order to improve the color of foods colorants are added to carbonated beverages, frozen desserts some dairy and baked products.

- **Color additives are used in foods**
  - **to offset color loss due to exposure to light, air, temperature extremes, moisture and storage conditions**
  - **to correct natural variations in color**
  - **to enhance colors that occur naturally; and**
  - **to provide color to colorless foods**
- **Natural – preferred by consumer**
- **Artificial – Safe and has excellent stability**

Flavouring agents

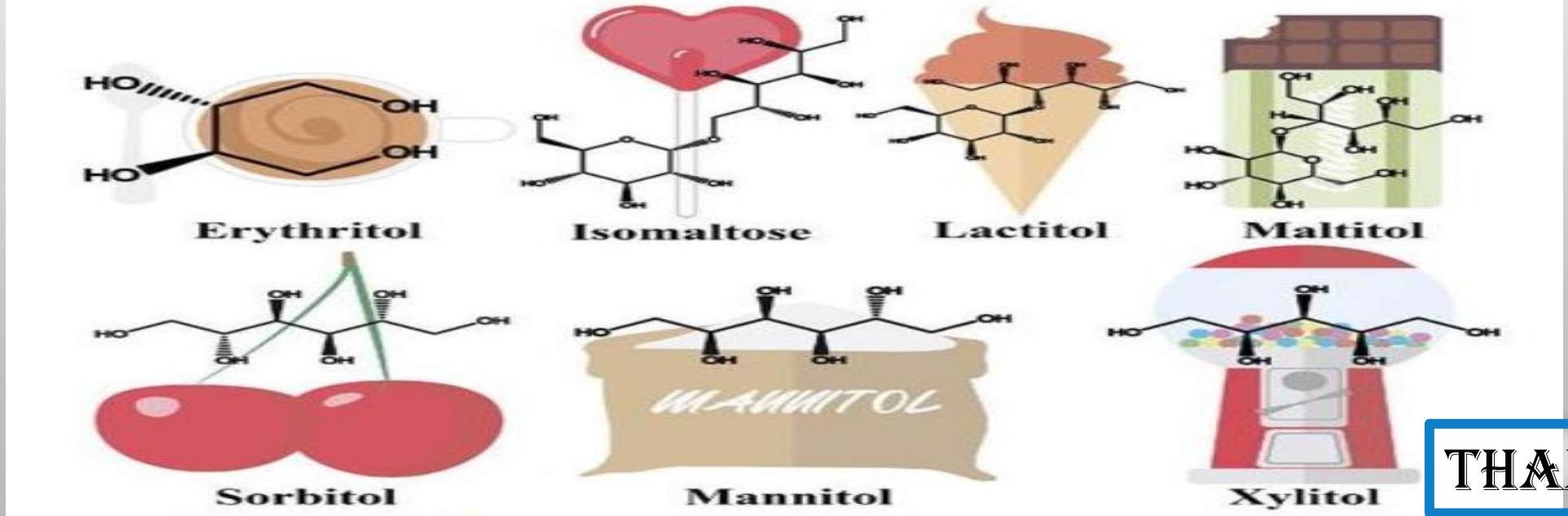
Natural food flavors are rarely used because the methods required to obtain the necessary amounts are expensive. Suppose that a manufactures wanted to produce bananas ice-cream, it will take 5 tons of bananas to extract ½ liter of banana oil. So, artificial flavorings become a necessity for fulfilling the demand for flavorings agents in our food. The flavoring agents commonly used are esters pentyl acetate responsible for banana flavor and benzyl aldehyde for cherry flavor.

Many flavouring agents possessing definite antimicrobial effects are used in foods. Flavor compounds generally have more antifungal activity than antibacterial. The non lactic, Gram positive bacteria are the most sensitive, and the lactic acid bacteria are rather resistant. The minimal inhibitory concentrations (MIC) of many flavouring compounds are 1,000 ppm or less against either bacteria or fungi. Many flavouring agents possessing definite antimicrobial effects are used in foods. Flavour compounds generally have more antifungal activity than antibacterial. The non lactic, Gram positive bacteria are the most sensitive, and the lactic acid bacteria are rather resistant. The minimal inhibitory concentrations (MIC) of many flavouring compounds are 1,000 ppm or less against either bacteria or fungi.

Sweeteners – It is added to many foods to enhance taste they can be classified as nutritive or non nutritive. Nutritive sweeteners contain calories because they are metabolized by the body to produce energy.

Nutritive sweetener include sucrose, glucose, dextrose, fructose and invert sugar, high fructose corn syrup. These high fructose corn syrup are produced from corn syrups, that have been treated with an enzyme, glucose isomerase's.

Non nutritive sweeteners, such as saccharin do not provide calories because they are not metabolized. Aspartame is classified as a non nutritive sweeter even though it is metabolized to two amino acids (phenylalamine and aspartic acid) because the level of aspartame used is extremely small but produce a sweetness equivalent to sucrose.



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