

## Food preservation by use of Chemicals

A large number of chemical have potential as food preservatives because of their ability to prevent/delay spoilage of foods caused by microorganisms and thus extend shelflife of food. Among several chemical substances only a few are permitted to be used in food products because of strict rules of safety by enforcement agencies and change in the antimicrobial property when incorporated to certain foods.

### Chemicals permitted in food as preservatives

Some of the chemicals having preservative effect are permitted to be used in foods and are generally recognized as safe (GRAS).

- Benzoic acid and parabens
- Sorbic acid
- Propionates
- Sulphur dioxide
- Nitrites and nitrates
- Antibiotics

#### 1. Benzoic acid and Parabens

Sodium salt of benzoic acid (sodium benzoate) was the first chemical preservative permitted in foods by FDA, and is widely used in large number of foods.

#### Characters

- ✓ Antimicrobial activity of benzoate is related to pH of food with greatest activity at low pH, and become ineffective as the pH increases towards neutral values.
- ✓ The antimicrobial activity of benzoates is due to the undissociated molecule and is greatest at low pH. About 60% is in undissociated form at pH 4, while only 1.5% remains as undissociated state at pH 6. Thus, it is most effective in low pH foods (fish sauce, tomatoe sauce, soft drinks etc).
- ✓ Effective against molds, yeasts and also on certain bacteria.
- ✓ 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%.

#### Parabens

Parabens are esters of para-hydroxy-benzoic acid and several forms of paraben are permitted in food. These include:

- methyl paraben
- heptyl paraben
- propyl paraben
- butyl paraben
- ethyl paraben

#### Characters

- ✓ Parabens differ from benzoates in antimicrobial activity as they are not affected by the pH of food.

- ✓ Parabens are effective under wide pH condition against both bacteria, yeasts and molds with Gram positive bacteria being more susceptible than Gram negative forms.
- ✓ Propyl paraben is most effective than methyl paraben for bacterial inhibition.
- ✓ Parabens are most effective against yeast and molds at 100-ppm level or less.
- ✓ The maximum permissible level in foods is 0.1%
- ✓ These are commonly used in soft drinks, bakery products, pickles etc.

## 2. Sorbic acid and sorbates

Sorbic acid is used as food preservation as salt of calcium, sodium and potassium. Maximum allowable level in foods is 0.20%.

### Characters

- ✓ Sorbates are most effective in acid foods (pH below 6) than neutral foods, and not effective in pH > 6.5.
- ✓ The undissociated form is responsible for antimicrobial activity, and about 86% is in undissociated form at pH 4.0 while, only 6% at pH 6.0.
- ✓ Effective against molds, yeasts and also wide range of bacteria. Inhibition of molds is due to the inhibition of dehydrogenase activity. Among bacteria catalase positive cocci are more sensitive than catalase negative forms. Also aerobes are more sensitive than anaerobes. These are found to be effective against *Staph aureus*, *Salmonella*, Coliforms, Psychrophilic spoilage bacteria, *Vibrio parahaemolyticus* and others.
- ✓ Sorbates are used to extend shelf life of products such as fresh fish, fresh poultry meat, perishable fruits, cheese, bakery products, fruit beverages etc.
- ✓ Also used along with nitrites in meat products to extend shelf life.
- ✓ Sorbates prevent growth of vegetable cells that are germinating from endospores.

### Mode of action of sorbates

Preservatives such as sorbates, benzoates and propionates are lipophilic compounds and antimicrobial activity is due to undissociated form. These chemicals affect proton motive force of bacterial cells. Being lipophilic, act on cytoplasmic membrane of microorganisms and separate proton (H<sup>+</sup>) and hydroxyl ions. The H<sup>+</sup> 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 aminoacids to inside cell, thus adversely affecting membrane transport and causing subsequent cell death.

## 3. Propionates

- ✚ Propionic acid and its calcium and sodium salts are permitted in foods as mold inhibitor in bread, cakes, cheese and other foods. Effective in low acid foods and commonly used in bread to prevent ropiness in bread dough.
- ✚ The inhibitory action is mainly fungistatic than fungicidal. Mode of action is similar to benzoates and sorbates. The undissociated form more effective (88% is in undissociated form at pH 4.0, and 6.7% at pH 6.0).
- ✚ Permissible limit in foods is 0.32%.

## 4. Sulphur dioxide and sulphites

Sulphur dioxide ( $\text{SO}_2$ ), sulphite ( $-\text{SO}_3$ ), bisulphite ( $-\text{HCO}_3$ ) and metabisulphite ( $-\text{S}_2\text{O}_5$ ) are used to control microorganisms and insects in foods such as molasses, dried fruits, wine, fruit juices etc.

$\text{SO}_2$  is used both as antimicrobial and agent and antioxidant. It is bacteriostatic at low pH condition at 100-200 ppm level and bacteriocidal at high concentration.

Aerobic microorganisms are more sensitive than fermentative forms. Yeasts are less sensitive than molds, acetic acid bacteria and lactic acid bacteria.

Molds (*Botrytis sp*) on grapes are controlled by periodic gassing with  $\text{SO}_2$ .

Bisulphites are used to destroy aflatoxins in foods and used at 200-300 ppm level.

### **Mechanism of action**

Actual mechanism is not known. But one of the possible explanation for the antimicrobial activity is the formation of undissociated sulphurous acid or gaseous sulphur dioxide, the effect of which is seen in low acid foods. Due to its strong reducing power lowers the oxygen tension below the level required by aerobic organisms, and by direct action on certain enzymes inhibits microorganisms. Metabisulphites affect vegetative cells during endospore germination.

### **5. Nitrites and Nitrates**

- ✚ Sodium nitrite and sodium nitrate are commonly used in meat curing because they help to stabilize red meat colour, inhibit spoilage and food poisoning organisms and contribute to flavour development.
- ✚ Many bacteria utilize nitrate and reduce it to nitrite. 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, nitrosomyoglobin. Nitrites are effective against several food poisoning (*Clostridium sp*) and spoilage microorganisms.

### **Period factor**

Formation of a substance or an agent which is ten times more inhibitory to *Clostridium spp* than nitrate alone when medium with added nitrite is heated is called perigo factor (named after the microbiologist Perigo, J A). Absence of botulism in cured, canned and vacuum packed meat and fish products is attributed to perigo factor. The inhibitory or antimicrobial effect results from heat processing or smoking of meat and fish products containing nitrite. Use of nitrite in these products is more for preventing food poisoning due to *Clostridium* rather than color and flavour development. Using at levels of 120 ppm causes antimicrobial effect, and 15-20 ppm helps in fixing colour and flavour development.

### **Made of action**

- Antimicrobial effect of nitrite is because of
- Inhibition of vegetative cell growth
- Preventing germination and growth of spores that survive heat processing/smoking during post processing storage.
- The antimicrobial effect of nitrite is due to its inhibition of non-heme, iron-sulphur enzymes. Inhibition of botulism by nitrite is due its effect on iron-sulphur enzymes thus preventing synthesis of ATP from pyruvate

## 6. Antibiotics

Antibiotics are secondary metabolites produced by microorganism such as fungi (Penicillium) and bacteria (Streptomyces, Actinomycetes). These inhibit/kill wide spectrum of microorganisms. Antibiotics are used extensively to treat, control and prevent human and animal disease. Use of antibiotics in foods to control spoilage organism started in 1950 with the use of tetracyclines in poultry. However, antibiotic use in food is not very popular because of risks involved.

### Factors to be considered while using antibiotic in foods

- ✚ Antibiotic agent should kill, and not inhibit the flora.
- ✚ Should ideally decompose in to harmless products
- ✚ Should be destroyed on cooking
- ✚ Should not be inactivated by food components or products of microbial metabolites
- ✚ Should not readily stimulate development of resistant strains
- ✚ Should not be used in food if used therapeutically or as animal feed additives.

### Antibiotics used in food

Some of the antibiotics used in food are;

- ✚ Tetracyclines
- ✚ Subtilin
- ✚ Tylosin
- ✚ Nisin
- ✚ Natamycin

### Tetracyclines

Among several antibiotics, chlorotetracycline (CTC) and oxytetracycline (OTC) are well suited to use in fresh foods. Tetracyclines are effective against microorganisms because they inhibit protein synthesis. CTC and OTC were approved as food preservatives in 1955 and 1956, respectively. These are heat sensitive and storage labile. Also used in human and animals to treat disease. These are used for extending shelf life of refrigerated fish and other seafoods, red meat, vegetables, raw milk and other foods. CTC is generally more effective than OTC in controlling spoilage flora. A dose of 7-10 ppm level is known to extend shelf life of refrigerated meat by 3-5 days. Use of CTC with sorbates extends shelf life of fish for up to 14 days. Antibiotics are used as feed supplement. Their use is restricted because the risks outweigh the benefits.

### Subtilin

Subtilin is produced by *Bacillus subtilis* and is effective against Gram positive bacteria. This is stable to acid treatment and heat resistant (stable at 121°C for 30-60 min). Subtilin is effective in canned foods at 5-20 ppm level in preventing germinating endospores. It is not used in human medicine and animal feed. The inhibitory effect of subtilin is because of its effect on membrane transport systems.

### **Tylosin**

Tylosin is effective against Gram positive bacteria. It inhibits protein synthesis by combining with 50S ribosomal subunit. Used mainly in animal feeds, and also to treat some diseases in poultry.

### **Nisin**

Nisin is a bacteriocin (not antibiotic) produced by some strains of *Lactobacillus lactis*, and is widely used in food preservation. Bacteriocins are small proteins which inhibit only closely related strains/species of Gram positive bacteria.

Nisin is effective against Gram positive bacteria and ineffective against fungi and Gram negative bacteria. The inhibitory effect is due to disruption of cytoplasmic membrane leading to pore formation, thus affecting membrane transport system.

Nisin is used in processed dairy foods. Use of nisin in low acid foods (vegetables) allows reduction in processing time and temperature. Used at 1% level in foods.

Nisin is desired in food as preservative because it is non toxic, produced naturally by lactic acid bacteria, heat stable, excellent storage stability, destroyed by digestive enzymes, does not contribute for off flavour/odour, and has narrow spectrum of antimicrobial activity.

### **Natamycin**

This antibiotic is isolated from *Strptomyces natalensis* and is effective against yeast and molds (antifungal). It is effective in controlling yeasts and molds at much lower concentration (1-25 ppm) than sorbates. These bind to sterols in cell membrane and disrupt selective membrane permeability.