COURSE TITLE: FOOD AND INDUSTRIAL MICROBIOLOGY
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FOOD PRESERVATION
NATURAL ANTIMICROBIAL COMPOUNDS

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PRESERVATIVES

According to FSSAI “Preservative” means a substance which when added to food, is capable of inhibiting, retarding or arresting the process of fermentation, acidification or other decomposition of food.

NEED

- Inhibit the growth of microorganisms like bacteria and fungi.
- Increase shelf life.
- Preserve the appearance of food
- Preserve the food characteristics like odor, taste and food is preserved for a long time

Classification of Natural preservatives

1. Plants with natural antimicrobial activities
2. Antimicrobials from microorganisms
3. Antimicrobials from animals

- Natural antimicrobials can derive from plants, animal sources, microorganisms, algae etc.
- Natural antimicrobial agents act as a viable and safer alternative to synthetic compounds.
- These natural antimicrobials ensure food safety without impairing organoleptic or nutritional properties of food products.
- Mode of action / mechanism: It creates rupture of microbial cell membrane, decay of proton motive force, interference with biomolecules' activity.
- The effect of natural antimicrobials on microorganism structure proved crucial for creating preservation effect of food items.

Natural antimicrobials

phenolics (flavonoids and non-flavonoids)

terpenes

isothiocyanates

alkaloids

antimicrobial peptides

chitosan

lysozyme

fatty acids and glycerides

nisin

natamycin

reuterin

pediocins

lactic acid

algae extracts

edible mushrooms extracts

Microbial agents

Staphylococcus aureus

Escherichia coli

Aspergillus flavus

Salmonella typhimurium

Listeria monocytogenes

Enterococcus faecalis

Bacillus cereus

Shigella dysenteriae

Clostridium tyrobutyricum

Candida albicans

Pseudomonas aeruginosa

Campylobacter jejuni

Penicillinum citrinum

Aspergillus parasiticus

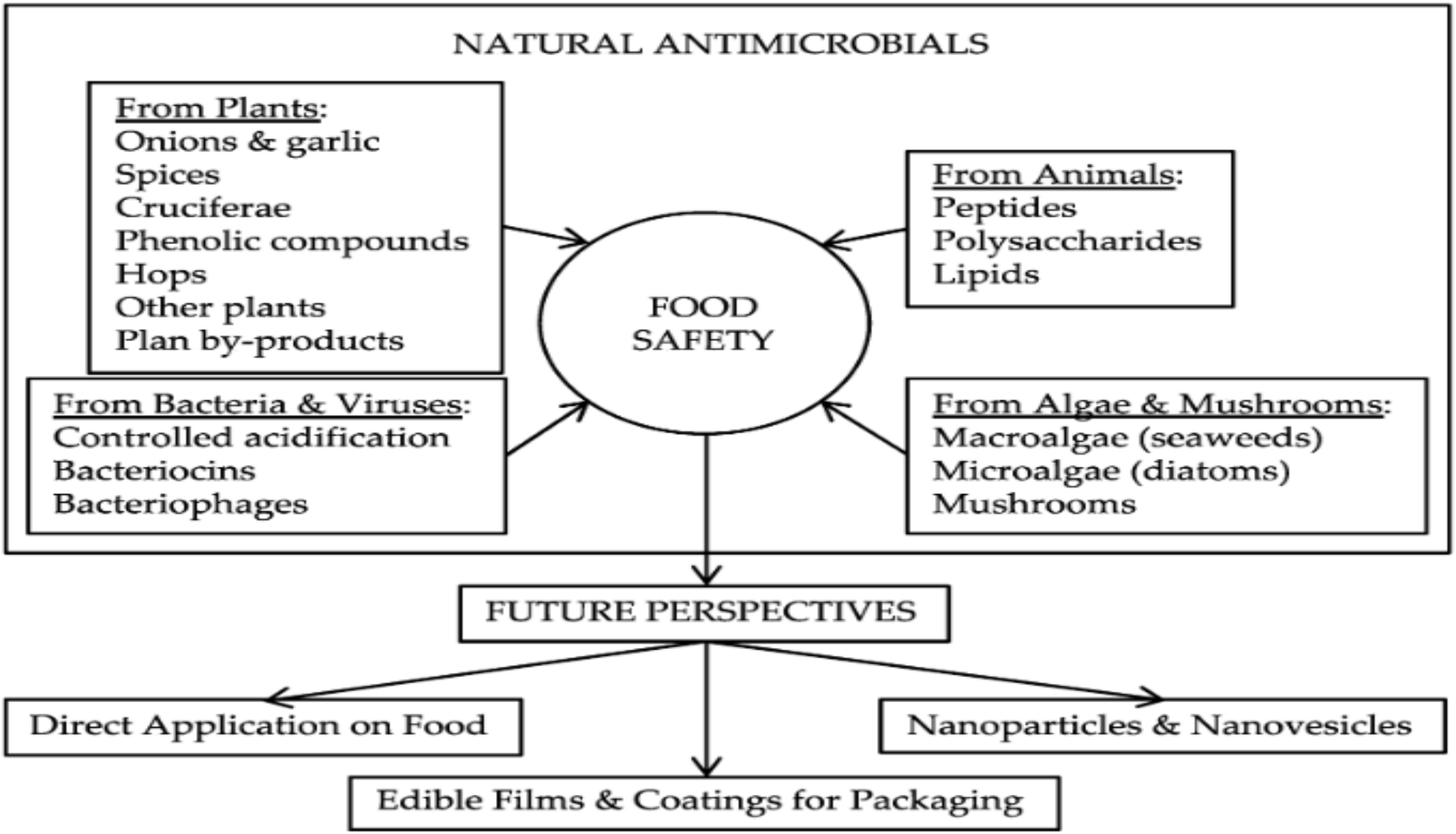
Saccharomyces cerevisiae

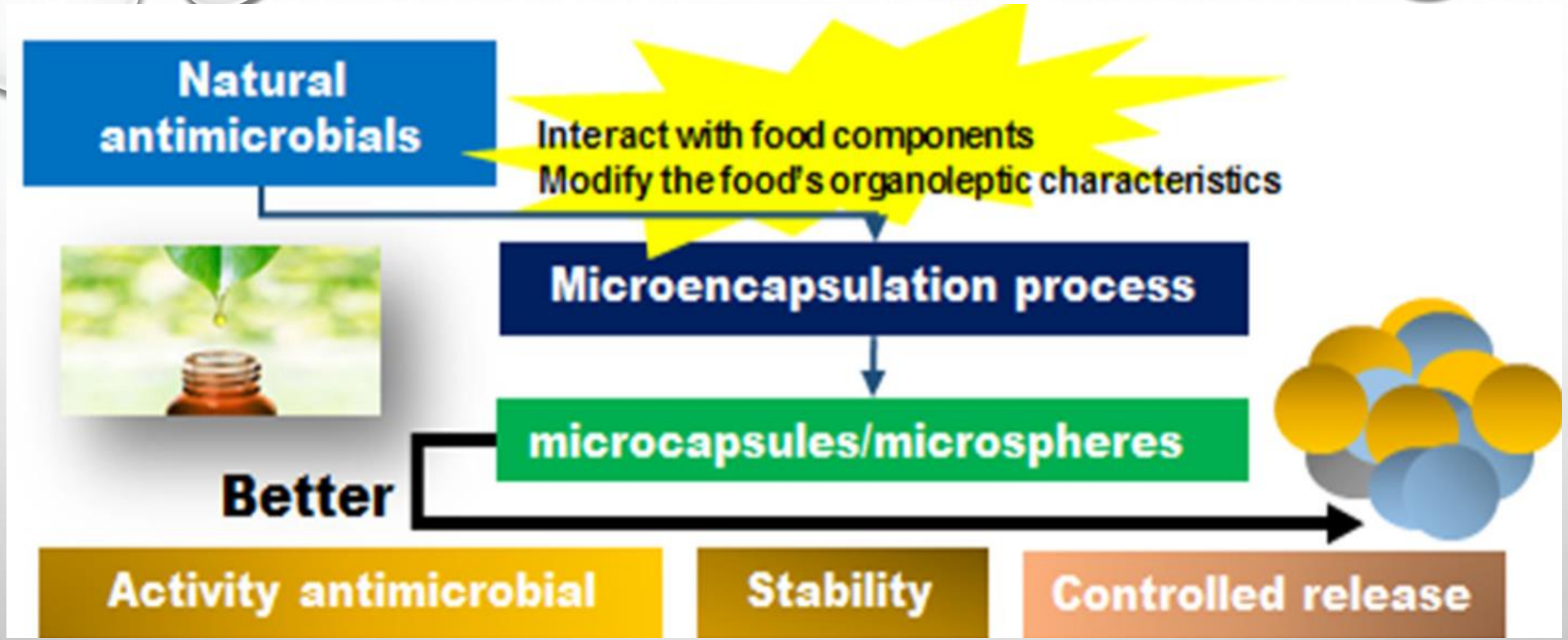
Effects

Microbial spoilage

Toxicity

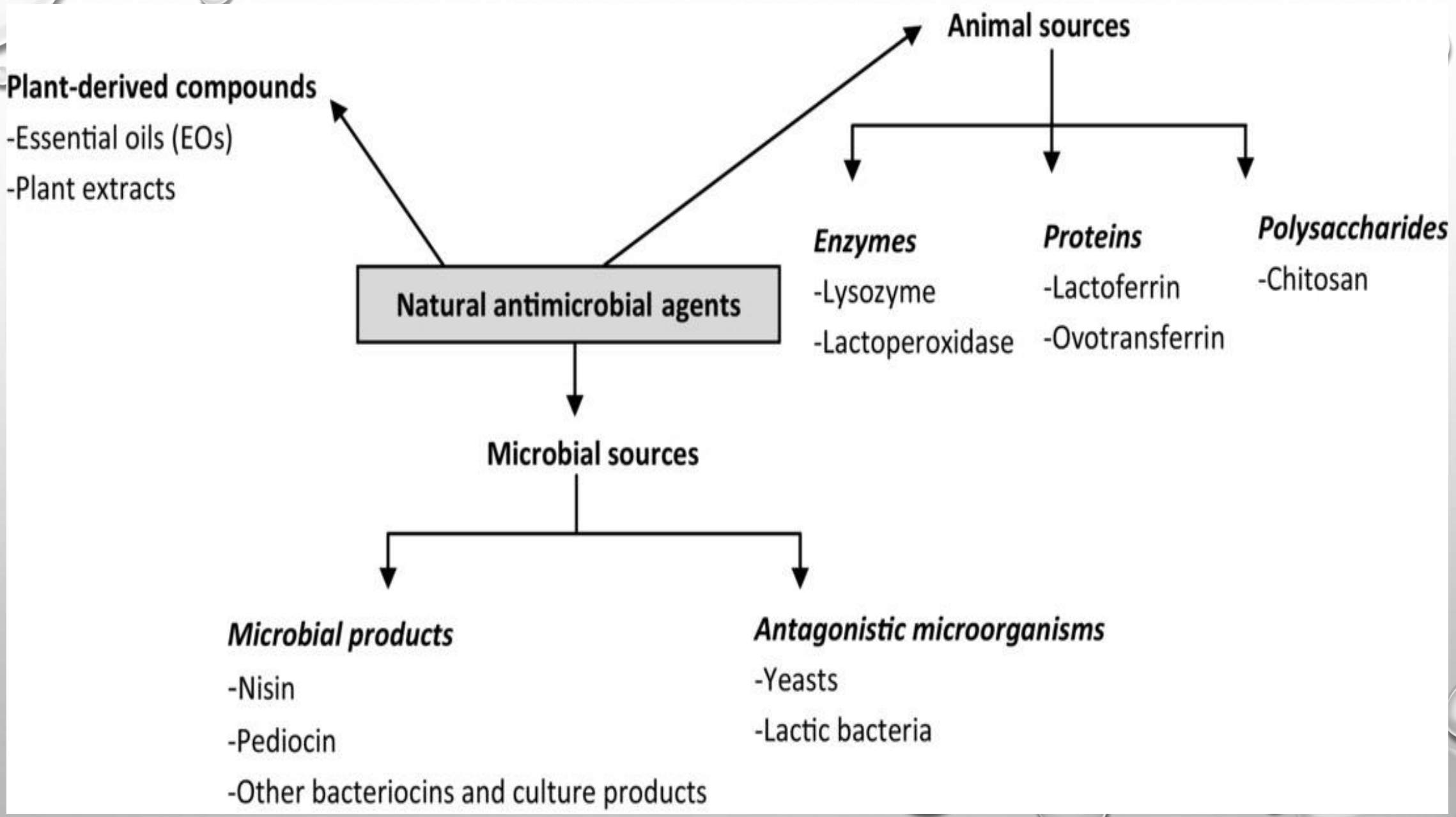
Organoleptic decay


NATURAL ANTIMICROBIALS
From Plants:
Onions & garlic
Spices
Cruciferae
Phenolic compounds
Hops
Other plants
Plan by-products
From Animals:
Peptides
Polysaccharides
Lipids
FOOD SAFETY
From Bacteria & Viruses:
Controlled acidification
Bacteriocins
Bacteriophages
From Algae & Mushrooms:
Macroalgae (seaweeds)
Microalgae (diatoms)
Mushrooms
FUTURE PERSPECTIVES
Direct Application on Food
Nanoparticles & Nanovesicles
Edible Films & Coatings for Packaging

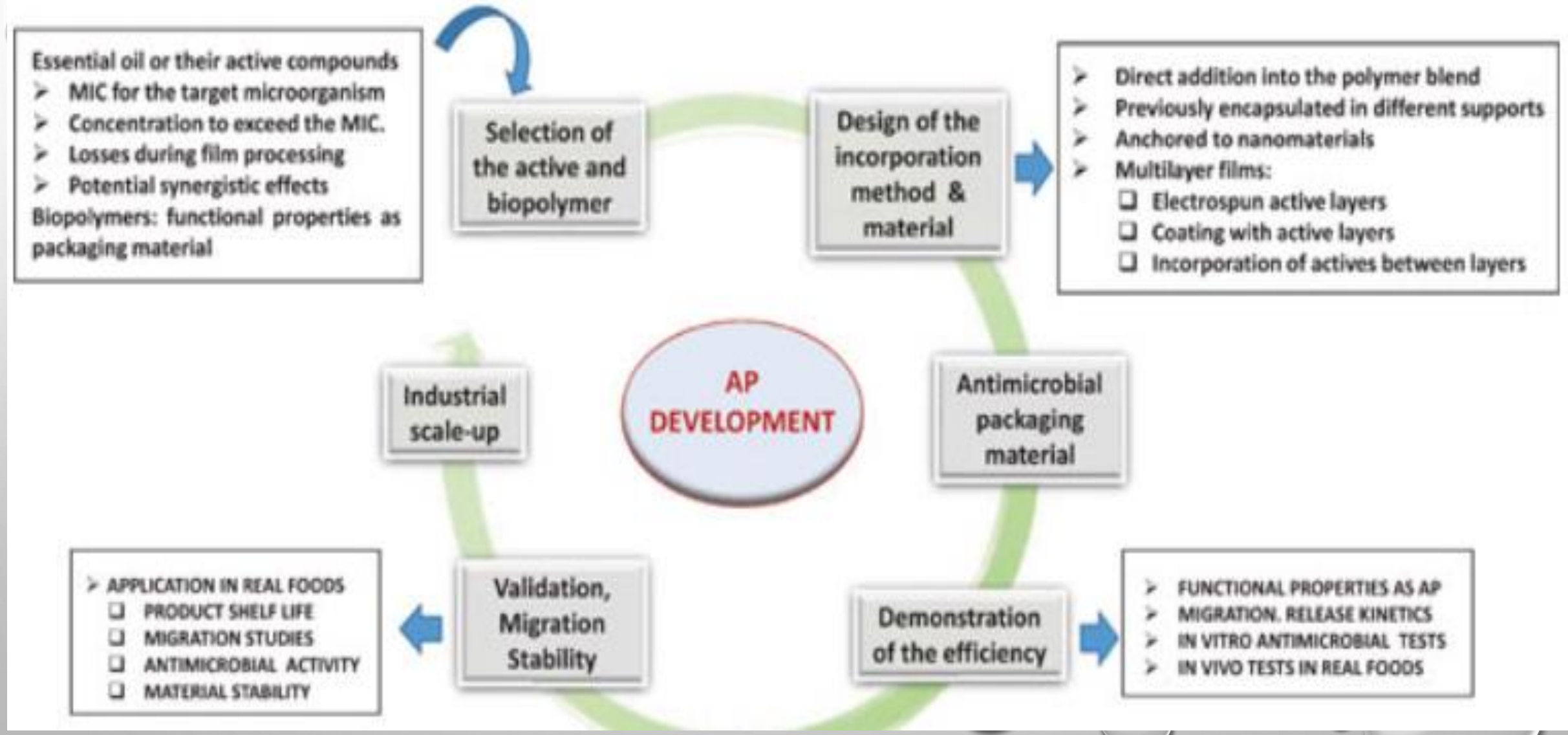


A novel trend is arising from health-conscious consumers expecting that natural antimicrobials act only against foodborne pathogens leaving the consumers' microbiome out of their scope. The negative effect of some synthetic preservatives on consumers' health is leading to more research to evaluate that natural antimicrobials fulfil food safety regulations

Antimicrobial Compound	Source	Advantages	Disadvantages
Chitosan	Shellfish	Inhibits bacteria and fungi Low toxicity	Low water solubility
Ovotransferrin	Egg whites	Inhibits bacteria and yeast	Common allergy
Lactoferrin	Milk	Inhibits bacteria and viruses	Needs to be purified
Lysozyme	Egg whites	Generally recognized as safe Low toxicity	Less effective against gram-negative bacteria (*Salmonella*, *E. coli*)
Cinnamic aldehyde	Cinnamon	Strong antimicrobial	Flavor consideration
Allicin	Garlic	Inhibits many microbes	Flavor and odor considerations
Nicin	*Lactococcus* bacteria	Effective against bacterial spores	Limited effect on gram negative bacteria and fungi
Natamycin	*Streptomyces* bacteria	Inhibits fungi Stable	Low water solubility


Plant-derived compounds
-Essential oils (EOs)
-Plant extracts
Animal sources
Enzymes
-Lysozyme
-Lactoperoxidase
Proteins
-Lactoferrin
-Ovotransferrin
Polysaccharides
-Chitosan
Natural antimicrobial agents
Microbial sources
Microbial products
-Nisin
-Pediocin
-Other bacteriocins and culture products
Antagonistic microorganisms
-Yeasts
-Lactic bacteria

Biopolymers & essential oils for antimicrobial packaging (AP)



VINEGAR (ACETIC ACID)

As per PFA vinegar should contain a minimum of 3.75% acetic acid.
Vinegar – manufactured by fermentation of fruit juice, potato and molasses by acetic acid bacteria and dilution of synthetic acid to the level of 3.75%.

ANTIMICROBIAL ACTION

- Lowers the pH of food products
- At pH < 3.5 : 10 to 100 times more powerful than other acids
- Penetrates bacterial cell wall and denatures the cell plasma proteins
- Reduce the heat sensitive bacteria
- Less action when the pH is > 5

Table Sugar and salt -- Sugar is used in making jellies and cured hams. Salt is also used in brines or sometimes it is directly applied to the food. Their effect is to increase osmotic pressure at a level which will prevent microorganism development. The cell growth of the micro organisms is inhibited or the organism itself may be completely destroyed. Same as sugar, salt also cause dehydration by drawing out and tying up water from the tissue of the food. Salt generally added to food also responsible for ionization yielding the chlorine ion, which is harmful to micro organisms and interferes with the action of proteolytic enzymes. The more salt used the greater the protection effect observed in the food.

Sugar preserving action depends upon the total sugar quantity in the finished product specially the total sugar concentration level in the liquid phase and a concentrations of 60% sugar in the finished product assures a good preservative affect on food.

Mechanism of action

- By osmosis water from fruits leached in to sugar solution and sugar enters to the fruit.
- Sugar reduce the solubility of oxygen in water.
- It decreases the water activity of food and inhibits the growth of microbes.

Activated lactoferrin (ALF, Activin)

An antimicrobial protein present in milk showed antibacterial, antifungal and anti viral activity. Also present in saliva, tears, and some other body fluids.

It has antimicrobial activity due to its capacity to chelate Fe^{2+} along with HCO^{-}_{3}. It binds to cell surfaces and has a high affinity for the outer membrane proteins (OMP) of Gram negative bacteria. It also inhibits growth and neutralizes endotoxins.

Ozone (O3)

This gaseous compound possesses antimicrobial activity. Just like chlorine, it is the most powerful oxidant available for conventional water treatment and is highly reactive. It is 1.5 times more potent than chlorine.

The cell target for O3 is the membrane where it disrupts permeability functions. Ozone is GRAS for bottled water use, and for use on a variety of fresh foods, but its strong oxidizing power does not recommend its use for red meats. It is effective against Gram positive and Gram-negative bacteria as well as viruses and protozoa. Ozone treatment can be used in vegetables, fruits, beef etc. to destroy pathogens like *E. coli* O157:H7, *S. typhimurium*, *Giardia lamblia*, etc.

Hydrogen peroxide (H_2O_2)

Hydrogen peroxide is a strong oxidizing agent and it is formed to some extent by all aerobic organisms, and it is enzymatically degraded by the enzyme catalase:

$2H_2O_2 \rightarrow 2H_2O + O_2$

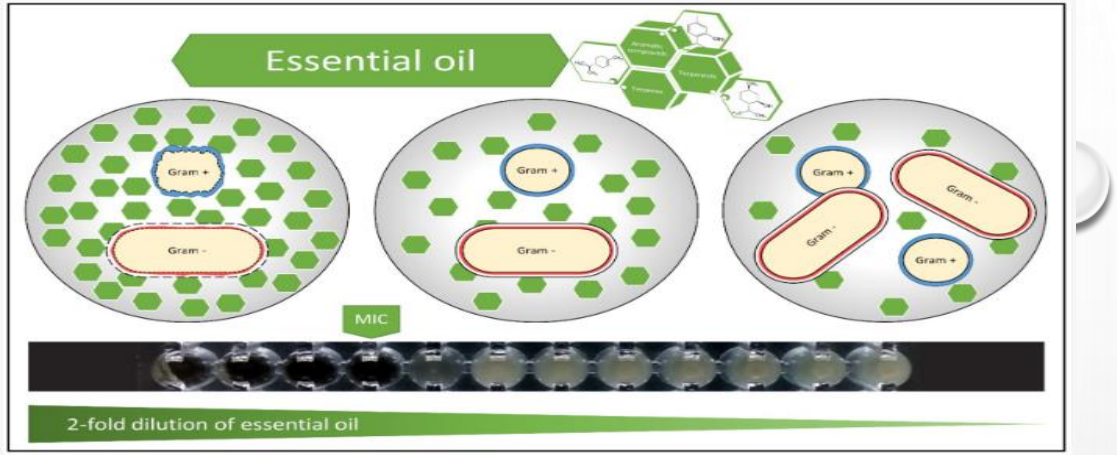
It is used as a sterilant for food contact surfaces of olefin polymers and polyethylene in aseptic packaging systems. Hydrogen peroxide vapours have microbiocidal properties. The antimicrobial effect of hydrogen peroxide attributes to a strong oxidizing effect on the bacterial cells and to the destruction of basic molecular structure of cellular proteins. H2O2 also prevent spores of *Bacillus cereus* from swelling properly during the germination process. It is used in the treatment of vegetables, fruits and fruit juice in the form of vapours.

Antimicrobial peptides

Antimicrobial peptides were first isolated from natural sources in the 1950s when nisin was isolated from lactic acid bacteria for potential application as a food preservative. Antimicrobial peptides are isolated from other natural sources, such as plants, insects, amphibians and marine organisms. Antimicrobial peptides (AMPs) are widely distributed in nature.

Spices and essential oils

Many spices possess significant antimicrobial activity. The antimicrobial activity is due to specific chemicals or essential oils, Such as eugenol and Allicin. Antimicrobial substances vary in content from the allicin of garlic (with a range of 0.3–0.5%) to eugenol in cloves (16-18%). Cinnamon and clove oils are also highly effective against *Aspergillus parasiticus* aflatoxin production. Plant EOs such as cumin, caraway and coriander have inhibitory effects on organisms such as *Aeromonas hydrophila*, *Pseudomonas fluorescens* and *Staphylococcus aureus*. Basil has high activity against *B. cereus*, *Enterobacter aerogenes*, *Escherichia coli*, and *Salmonella*. Lemon balm showed adequate activity against *L. monocytogenes* and *S. aureus*. Oregano had comparatively high activity against enterobacteria.



Effects of essential oil on bacteria. Gram-positive peptidoglycan cell wall allows hydrophobic molecules to penetrate and reach the internal environment. The lipopolysaccharide, which is part of the external layer of Gram-negative bacteria, allows mainly small hydrophilic molecules to pass and is only partly permissive for hydrophobic molecules. The hydrophobicity of essential oils is responsible for the disruption of bacterial structures. The mechanisms of action of essential oil on bacteria are: degradation of the cell wall and cytoplasmic membrane, cytoplasm coagulation and diffusion through the double lipid layer of the membrane, together with alteration of its permeability and function.

Herbal spices

It acts as antimicrobials and these are the homogenous group of substances extracted from various plant parts. eg :Turmeric – curcumin, Pepper – piperin, Clove – eugenol

Sr.no	Spices and herb	Inhibitory effect
1	Cinnamon, clove,mustard	Strong
2	Allspice, coriander, cumin	Medium
3	Black pepper, chilli powder, ginger	weak

MECHANISM OF ACTION It damages the membrane integrity of microorganisms and causes leakage of ions, ATP, nucleic acids and amino acids from microorganisms. It also affects pH of the microorganisms when used at high concentration.

Antioxidants

Antioxidants are beneficial in preventing rancidity in fats and foods containing fats. When food containing fats exposed to light, moisture, heat or heavy metal ions it becomes activated and oxidize (reach with available oxygen) to peroxides.

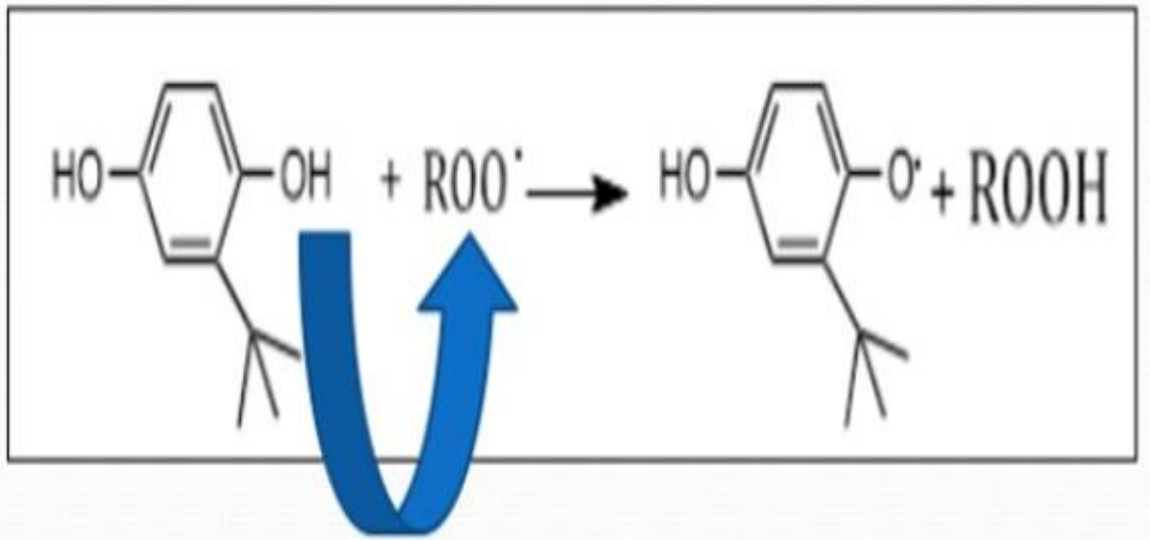
The most used antioxidants are Butylated Hydroxy Anisole (BHA), Butylated Hydroxy Toluence (BHT), Propyl Gallate, Natural / Synthetic Tocophelos (Vitamin E) Ascorbic Acid (vitamin C) and Lecithin. BHA + BHT are frequency used in variety of products because they are relatively stable to heat and maintain their effect in cooked products.

Antioxidant compounds	**Foods containing high levels of these antioxidants**
Vitamin C (ascorbic acid)	Fresh Fruits and vegetables
Vitamin E (tocopherols, tocotrienols)	Vegetable oils
Polyphenolic antioxidants (resveratrol, flavonoids)	Tea, coffee, soy, fruit, olive oil, chocolate, cinnamon, orega no and red wine
Carotenoids(lycopene, carotenes, lutein)	Fruit, vegetables and eggs.

Synthetic antioxidants	Natural antioxidants
Inexpensive	Expensive
Widely applied	Use restricted to some products
Medium to high antioxidant activity	Wide ranging antioxidant activity
Increasing safety concern	Perceived as innocuous substance
Use banned for some of them	Increasing use and expanding applications
Low water solubility	Broad range of solubility
Decreasing interest	Increasing interest

Antioxidants slow down the oxidation rates of **foods** by a combination of scavenging free radicals, chelating prooxidative metals, quenching singlet oxygen and photosensitizers and inactivating lipoxygenase.

Antioxidant function:
Transfer of e^- from phenol group.



Antibiotics

These are the secondary metabolites produced by microorganism such as fungi (*Penicillium*) and bacteria (*Streptomyces*, *Actionmycetes*). They inhibit / kill wide spectrum of microorganisms. Antibiotics are extensively use to treat, control and prevent human and animal disease but in foods it is used mainly to control spoilage organism. Its use in food started in 1950 with the use of tetracyclines in poultry.

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.

Thank You