Detection of spoilage and characterization

End Products From Microbial Metabolism Of Food Nutrients

- **Carbohydrate compounds – CO_2, H_2, H_2O_2, Lactate, Acetate, Formate, Succinate, Butyrate, Ethanol, Propanol, Butanol, Diacetyl, Dextran**
- **Proteins and Non Protein Nitrogen compounds – CO_2, H_2, NH_3, H_2S, Amines, Ketoacids, Putrescines**
- **Lipids – Fatty acids, Glycerol, Hydroperoxides, Aldehydes, Ketones**

Indicators Of Microbial Food Spoilage

Indicators which can predict expected shelf life and estimate stages of microbial food spoilage

- **Sensory indicators…….change in color, odor, flavor, texture and general appearance**
- **Microbiological Indicators…..types of microorganisms causing spoilage**
- **Chemical indicators……..specific microbial metabolites**

The contributing factors in microbial spoilage of a food

- The type of a product
- Its composition
- Methods used during processing
- Contamination during processing
- Nature of packaging
- Temperature and time of storage
- Possible temperature abuse

Microbiological Indicators

1. Enumeration of colony-forming units(CFUs) - Select the microorganisms predominantly involved in spoilage of a food(or food group) as indicators of spoilage for that food
2. Aerobic plate count(APC), or standard plate count(SPC) indicate the effectiveness of sanitary procedures used during processing and handling and before storage of a product
3. Determination of lipopolysaccharides(LPS) in a food(for Gram-negative bacteria)
4. Measurement of ATP as its concentration is increased with high numbers of viable cells

Examples

- **Refrigerated raw (fresh) meats stored aerobically - Enumeration of CFUs of psychrotrophic aerobes especially Gram-negative aerobes Incubation temperature 10-25^{0}C**
- **Refrigerated raw(fresh) meats stored anaerobically (vacuum packaged) - Enumeration of CFUs of psychrotrophic lactic acid bacteria and Enterobacteriaceae under anaerobic conditions**
- **Raw Milk - SPC, psychrotrophic Gram-negative bacteria and thermoduric bacteria**
- **Pasteurized Milk - SPC, psychrotrophic bacteria both Gram-negative and Gram-positive**
- **Butter - Lipolytic microorganisms**
- **Salad Dressing - Lactobacillus Spp.**

Chemical Indicators

As microorganisms grow in foods, they produce many types of metabolic by-products associated with the spoilage characteristics:

1. Microbial Products:

H_2S, NH_3, CO_2, Diacetyl, Acetoin, Indole, changes in pH

- **Biosensors may be developed that could be effective for indicating changes in specific metabolites by a group of bacteria**

2. Microbial Enzymes: Proteinases of some psychrotrophic bacteria such as *Pseudomonas fluorescens*

- **ELISA and Flurescamine assay - It reacts with amino acids to form a fluorescent compound at pH 9.0 and measured fluorimetrically to determine protein hydrolysis**
- **Trinitrobenzene sulfonic acid(TNBS) - It reacts with free amino groups and develops color that can be colorimetrically measured to determine the amount of free amino acids present because of proteolysis**
- **Heat-Stable Lipases in Milk - Milk is heated to destroy milk lipases but not the bacterial heat-stable lipases**

Characterization of Microorganisms

A. Bacteria

Morphological Characteristics:

- Examination to ascertain the shape, size, aggregation, structure and staining
- Encapsulation: The presence of capsules (made up of polysaccharides of dextrin, dextran or levan) may account for sliminess or ropiness of a food
- Formation of Endospores: Bacteria of genera *Bacillus, Clostridium, Sporosarcina* etc have the ability to form endospores
- Formation of Cell Aggregates: It is characteristic of some bacteria to form long chains or of others to clump under certain conditions. It is more difficult to kill all bacteria in intertwined chains or sizable clumps than to destroy separate cells

Cultural Characteristics:

- Bacterial growth in and on foods often is extensive enough to make the food unattractive in appearance or otherwise objectionable
- Pigmented bacteria cause discolouration on the surfaces of foods; films which may cover the surfaces of liquids; growth may make surfaces slimy; or growth throughout the liquids may result in undesirable cloudiness or sediment

Physiological Characteristics

Most bacteria may be placed into one of three groups based on their response to gaseous oxygen (Aerobic , anaerobic and facultative anaerobes)

Bacteria may also be classified both by the mode by which they obtain their energy (heterotrophs; including Saprotrophs and autotrophs; including photoautotrophic, chemoautotrophic)

B. Molds

Morpohological Chactacteristics:

- The morphology including the form and structure, of molds, as judged by their macroscopic and microscopic appearance
- Hyphae and Mycelium: The mold thallus consists of a mass of branched, intertwined filaments called hyphae, and the whole mass of these hyphae are known as the mycelium
- Reproduction of molds is chiefly by means of asexual and sexual spores

Culture Characteristics:

The gross appearance of a mold growing on a food often is sufficient to indicate its class or order

Some molds are loose and fluffy; others are compact

Some look velvety on the upper surface, some dry and powdery, and others wet or gelatinous

Some molds are restricted in size, while others grow by the food or container

Pigments in the mycelium – red, purple, yellow, brown, gray black, as the pigments of mass of asexual spores

Physiological Characteristics:

- **Moisture Requirements: In general most molds require less available moisture**
- **Temperature Requirements: Most molds would be considered mesophilic, but some grow well at 35 to 37°C or above, and some at still higher temperatures (thermophilic) or very low temperatures (psychrotophic or**
- **Oxygen and pH Requirements: Molds are aerobic; i.e. they require oxygen for growth similarly, most molds can grow over a wide range of hydrogen-ion concentration (pH 2 to 8.5), but the majority are favoured by an acid pH**
- **Food Requirements: Molds in general can utilize many kinds of foods, ranging from simple to complex**
- **Inhibitors: Compounds inhibitory to other organisms are produced by some molds, such as penicillin from Penicillium chrysogenum and clavacin from Aspergillus clavatus and certain chemical compounds are mycostatic, inhibiting the growth of molds (sorbic acid, propionates, and acetates are examples), or are specifically fungicidal, killing molds**

C. Yeasts

- **The term refers to those fungi which are generally not filamentous but unicellular and ovoid or spheroid and which reproduce by budding or fission**
- **Yeasts being useful or harmful in foods, are involved in the fermentations and manufacture of foods such as bread, beer, wines, vinegar, and surface-ripened cheese, and also grown for enzymes**
- **Yeasts are undesirable when they cause spoilage of sauerkraut, fruit juices, syrups, molasses, honey, jellies, meats, wine, beer, and other foods**

Morphological Characteristics:

- **Form and structure: The form of yeasts may be spherical to ovoid, lemon-shaped, pear-shaped, cylindrical, triangular, or even elongated into a false or true mycelium with different sizes**
- **Reproduction: Most yeasts reproduce asexually by multilateral or polar budding; some new species or yeasts reproduce by fission, and one reproduces by combination of fission and budding**
- **Sexual reproduction of "true" yeasts (Ascomycotina) results in the production of ascospores with different colour, smoothness or roughness of their walls, and in their shape (round, oval, reniform, bean or sickle-shaped, hemispherical, angular, fusiform, or needle-shaped)**

Cultural Characteristics:

- **Massed yeast growth is not useful in the identification, although growth as a film on the surface of liquid media suggests an oxidative or film yeasts, and production of a carotenoids pigment indicates the genus *Rhodotorula***
- **Yeasts are oxidative, fermentative, or both. The oxidative yeasts may grow as a film, pellicle, or scum on the surface of liquid, fermentative yeasts usually grow throughout the liquid and produce carbon dioxide**

Physiological Characteristics:

- Most common yeasts grow best with a plentiful supply of available moisture
- The range of temperature for growth of most yeasts is, in general, similar to that for molds, with the optimum around 25°C to 30°C and the maximum about 35°C to 47°C
- The growth of most yeasts if favoured by an acid reaction in the vicinity of pH 4 to 4.5, and they will not grow well in an alkaline medium unless adapted to it
- Yeasts grow best under aerobic conditions, but the fermentative types can grow anaerobically, although slowly
- Sugars are the best source of energy for yeasts, although oxidative yeasts, e.g., the film yeasts, oxidize organic acids and alcohol. Carbon dioxide produced by bread yeasts accomplishes the leavening of bread, and alcohol made by the fermentative yeasts is the main product in the manufacture of wines, beer, industrial alcohol, and other products. The yeasts also aid in the production of flavors or “bouquet” in wines