Principles of Microbiological Testing
Methodological Concepts

BY

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FOOD & FOOD PROCESSING

Food processing is the set of methods and techniques used to transform raw ingredients into food.

COMMON FOOD PROCESSING TECHNIQUES ARE:-

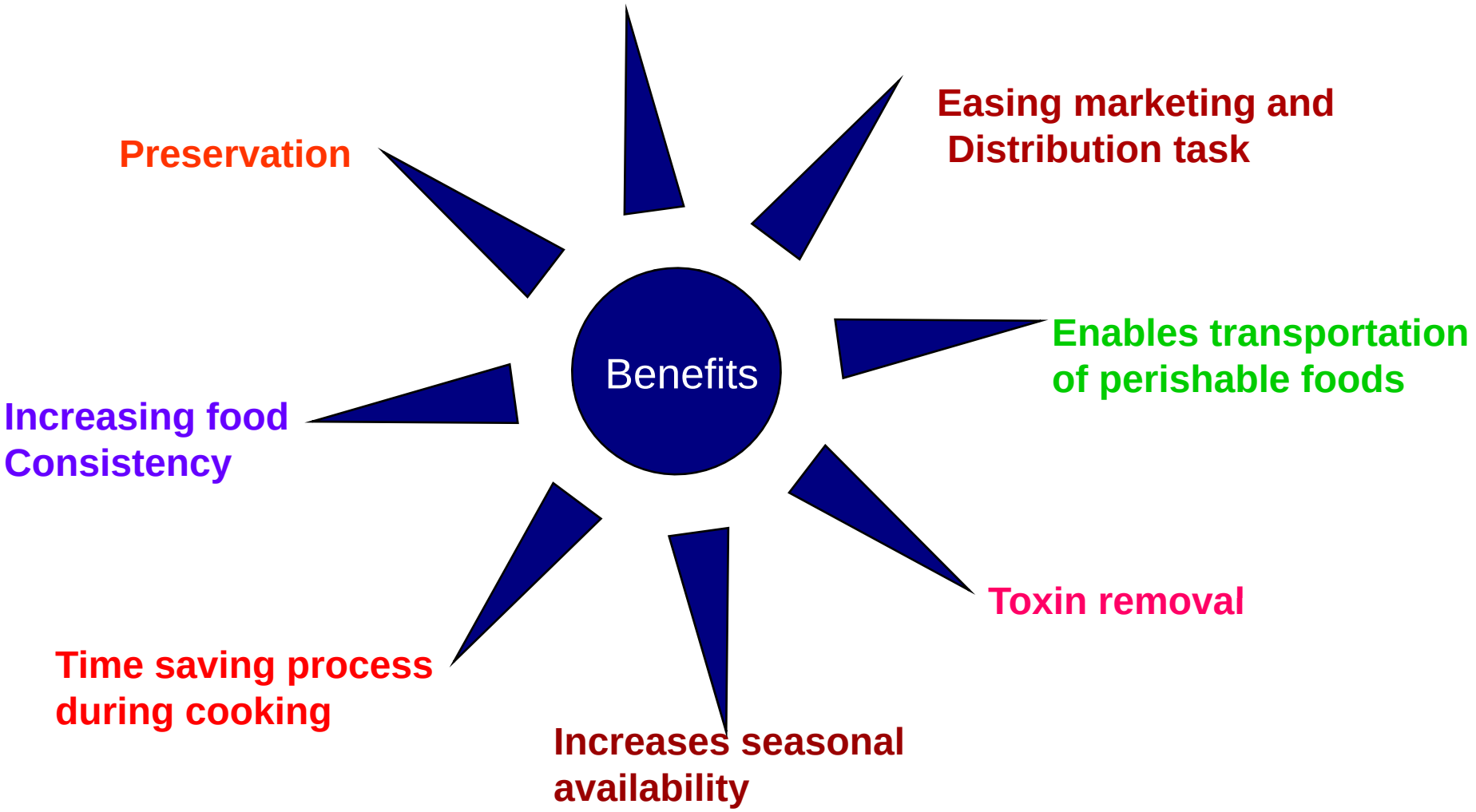
- **Cooking, such as boiling, frying, steaming or grilling**
- **Pasteurization**
- **Liquefaction, such as to produce fruit juice**
- **Fermentation. e.g. in beer breweries**

INDIAN COOKED FOOD

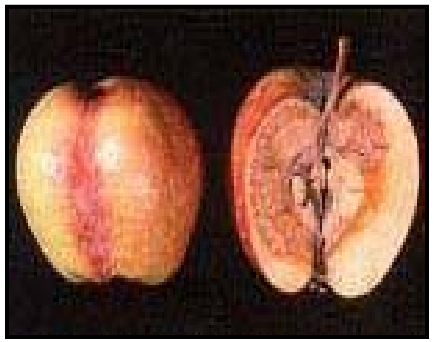
INDIAN MUGHLAI FOOD

PROCESSED FOOD

BENEFITS OF FOOD PROCESSING



SPOILED FOOD & FRUITS



Classification of foods by ease of spoilage

1. **Stable / non-perishable foods:** e.g. sugar, flour – not spoiled unless handled carelessly.

2. **Semi-perishable foods:** e.g. potatoes, apples – if properly handled and stored, remain unspoiled for a long period.

3. **Perishable foods:** e.g. meats, fish, poultry, eggs and milk – daily foods that spoil readily unless special preservative methods are used.

How does food processing influence the spoilage rate and the type of spoilage that occurs?

1. Increase in the number of microorganisms during processing.

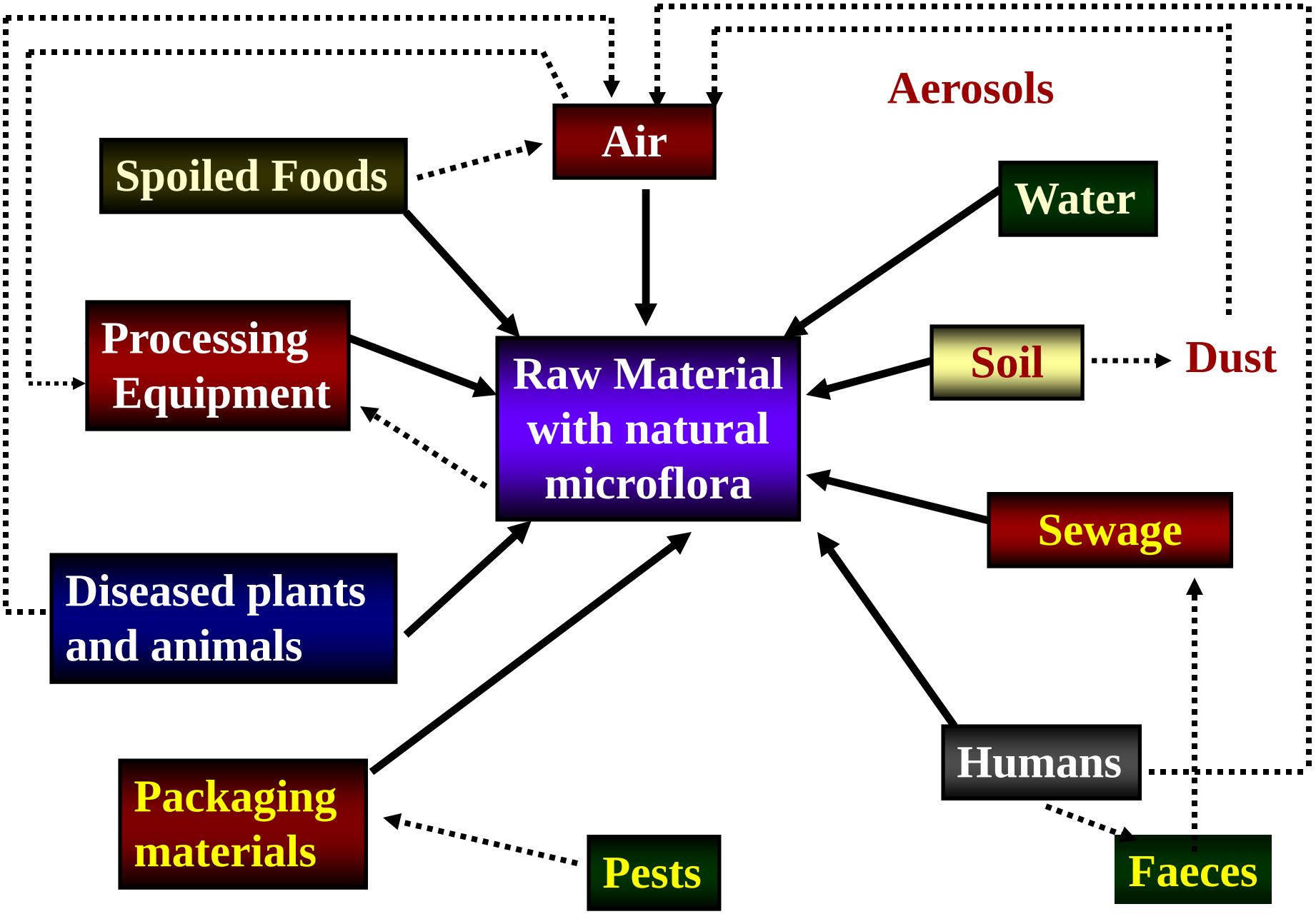
2. Destruction of the normal spoilage flora and the introduction of a new microflora.

3. Changes in the intrinsic and extrinsic parameters of the food.

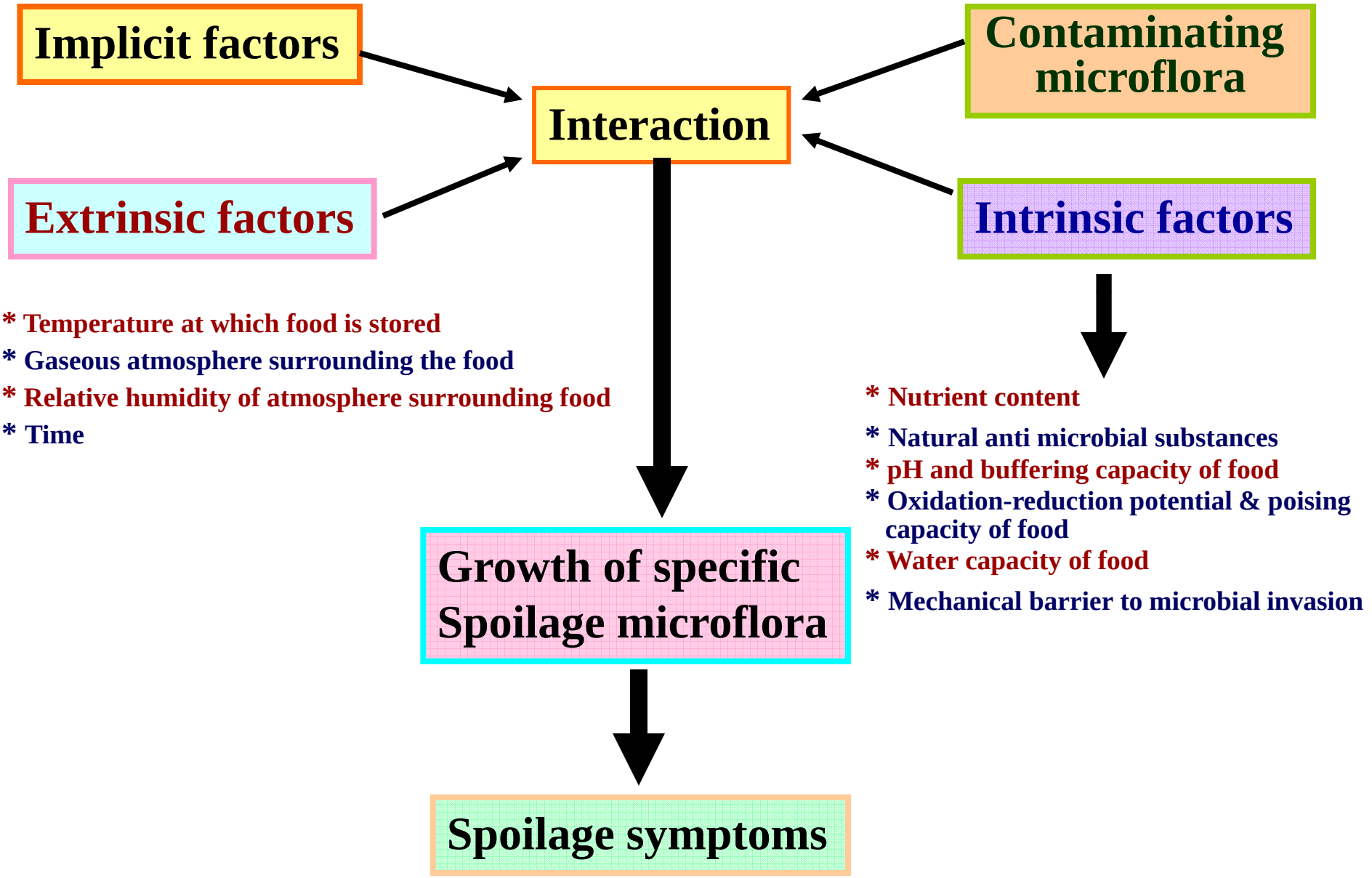
Major reasons for a food being rejected as spoiled

1. Organoleptic changes brought about by the growth of microorganisms.
2. Chemical changes in a food e.g. oxidative rancidity of fats, browning of fruits and vegetables.
3. Physical changes e.g. burning, drying, pressure, freezer burn etc.
4. Staling due to changes in H_2O content giving a change in texture e.g. stale baked products.
5. Over Ripening overripe fruits are considered spoiled.
6. Contamination with chemical agents e.g. sanitizers that give rise to unacceptable odours and flavors.
7. Microorganisms and Insects.

Sources of contamination of food



Interactions involved in the selection of a spoilage microflora

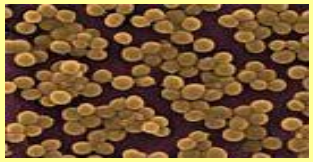
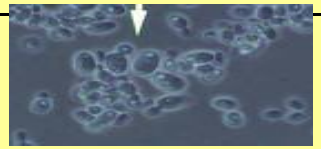


Composition of the contaminating microflora

The number of different types of microorganisms contaminating a food material can be large.

- **Gram-negative rods and coccobacilli:** ***Acinetobacter, Aeromonas, Alkaligenes, Citrobacter, Escherichia, Flavobacterium, Moraxella, Proteus, Pseudomonas, Salmonella, Yersinia, Shewanella, Enterobacter***
- **Gram-positive rods:** ***Bacillus, Brochothrix, Clostridium, Corynebacterium, Lactobacillus*** **and** ***Listeria***
- **Gram-positive cocci:** ***Enterococcus, Lactococcus, Micrococcus, Pediococcus*** **and** ***Staphylococcus***
- **In addition, a dozen or so species of molds, the most common of which are the mucoraceous types** ***Mucor, Rhizopus*** **and** ***Thamnidium*** **and the imperfect fungi** ***Penicillium, Cladosporium, Geotrichum*** **and** ***Sporotrichum.***
- **About 6 genera of yeast are known to contaminate meat. The most common is** ***Candida*** **spp.**

Most common microorganisms which spoil food

Organism	Food involved
Clostridium perfringens 	Contaminates poultry meat and meat products, especially stews, gravies and pies.
Salmonella 	Contaminates poultry meat and meat products, especially poultry. custard, cream, milk and egg products and salads.
Staphylococcus 	Contaminates moist protein foods. Meat, eggs and fish products.
Yersinia 	Contaminates meat and meat products, especially pork mince and tongue. Contaminated water, seafood and raw milk
Yeasts 	Sweet, acidic refrigerated foods or jams /jellies.

MICROBIOLOGICAL CRITERIA OF FOOD SPOILAGE

A microbiological criterion defines the limit above which a food is considered to be contaminated at an unacceptable level with a microorganism/s.

A microbiological criterion contains the following components:

- The sampling plan (the number of samples of a food that should be taken).
- The laboratory method (the method which should be used to test the food).
- The stage in the food chain where the criterion applies.
- The corrective action to be taken by Food Business Operator (FBO) when the criterion is not met.

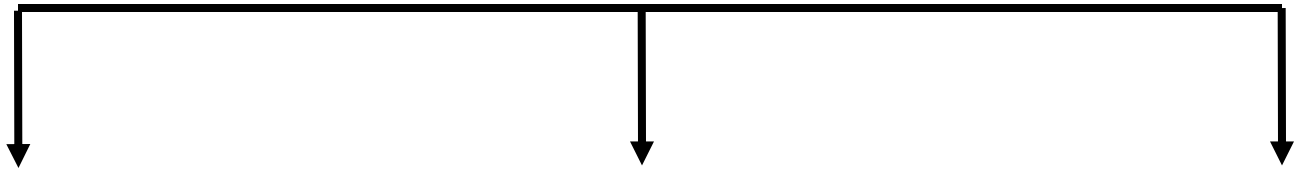
DETECTION OF MICROBIAL CONTAMINATION OF FOOD AND FOOD PRODUCTS

Over the last 70 to 80 years, many different methods have been developed for detecting pathogenic microorganisms or their toxins in food or in food products.

Identification and typification of microorganisms is NECESSARY AND IMPORTANT for :-

- **Prevention, diagnosis and treatment of contagious illnesses**
- **Determination of the source of pollution of the environment**
- **Assessment of risk to public health**
- **Evaluation of epidemiology**

METHODS OF DETECTION OF MICROBIAL CONTAMINATIONS IN FOOD OR FOOD PRODUCTS



A) CONVENTIONAL METHODS

- Plate count
- Membrane filtration
- Most probable number
- Dye reduction tests
- Indicators

B) RAPID METHODS

- ATP photometry
- Direct Epifluorescent Filter Techniques (DEFT)
- Electrical impedance methods
- Immuno assay
- Flow cytometry
- Impedimetry

C) MOLECULAR METHODS

- Polymerase Chain Reaction (PCR)
- Immunofluorescence
- Blotting

CONVENTIONAL METHODS

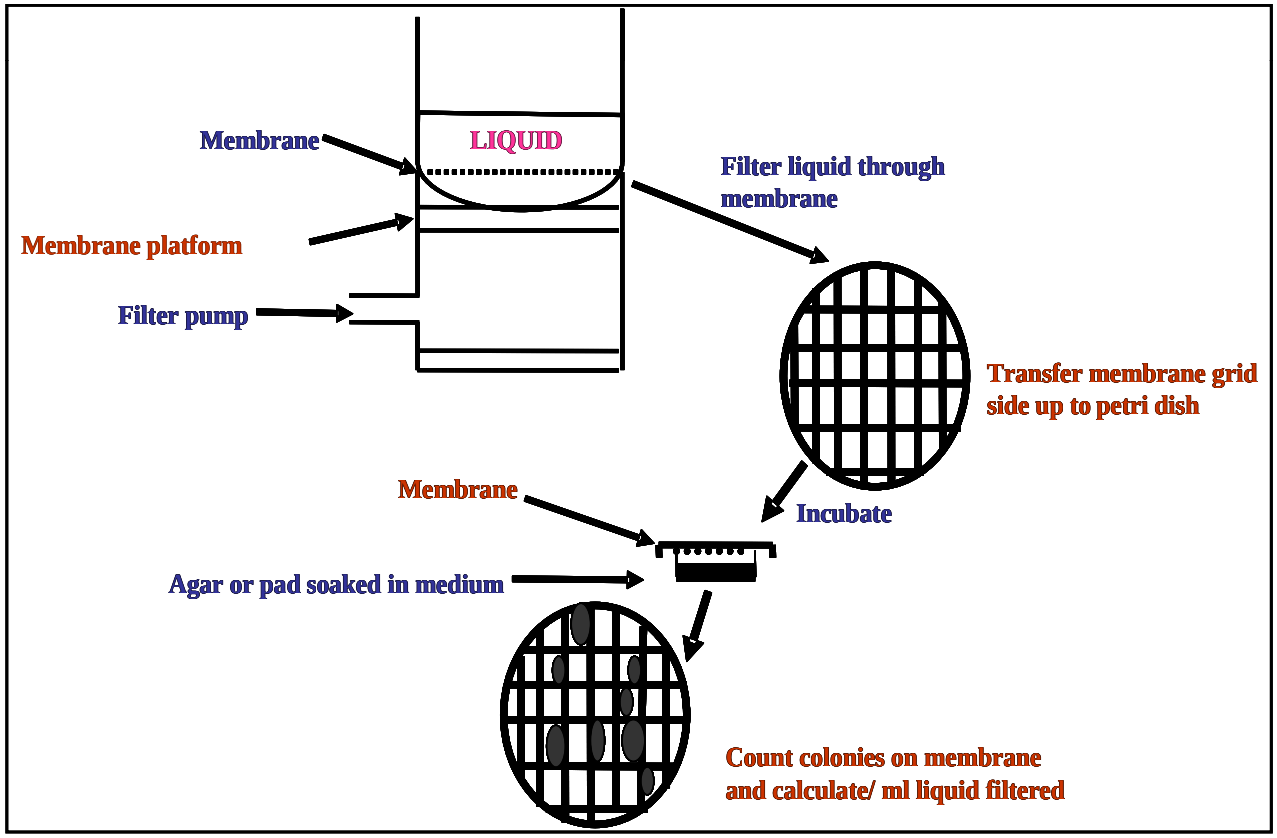
Plate count

The information we get, from a plate count depends on the following:

- **The choice of diluents used to prepare the food homogenates and the dilution series**
- **The medium used**
- **The plating methods**
  - i) Spread plate
  - ii) Pour plate
- **Incubation conditions**
- **Temperature**
  - i) Gaseous atmosphere
  - ii) Time
- **Method used to homogenize the sample**

Membrane filtration technique

- Involves passing a known volume of liquid through a cellulose acetate membrane with a pore size of 0.45 mm.
- Use is limited to clear liquids that do not contain debris or other materials that will block the filter.



Most Probable Number (MPN) technique

- The interpretation of the MPN method was easier, the pattern of growth was observed visually and then this pattern was compared with standardized MPN table. It means the MPN method offered an economic way from time and effort.

- MPN method gives more benefit in colony counting because the broth can be incubated for a longer time and still allows an accurate determination of the colony count.

- Reduction of working time and material as a rising of trial quantity and reduction of substance doses needed for testing.
- Particularly useful for indicators e.g. *E.coli and Staphylococcus aureus*

Dye reduction test

- Use of redox dyes (e.g. methylene blue and resazurin to assess milk quality) is based on the fact that the micro flora present in the milk will metabolize carbohydrates to produce reducing substances that in turn reduce the dye.

- The resazurin test is applied as a quick test to assess the quality of bulk tanker milk before it is accepted at the dairy (platform rejection test).

1 2 3 4 5

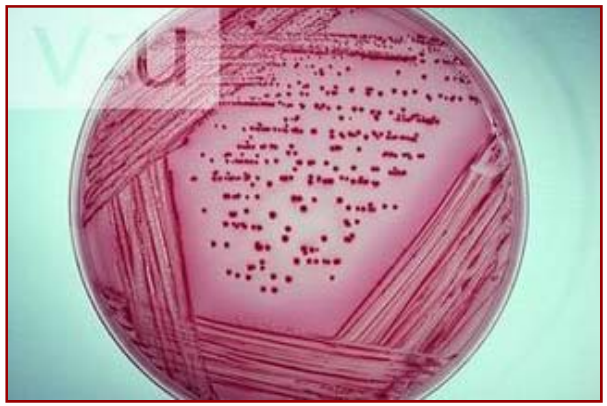
Test for the quality of milk (Resazurin test)

1. Blue (no color change) : Excellent
2. Blue to deep mauve : Good
3. Deep mauve to deep pink : Fair
4. Whitish pink : Poor
5. Deep pink : Poor

Indicators

- **The idea of using indicator organism to assess food quality originated from water microbiology.**

- **Source laboratories now test foods for total *Enterobacteriaceae* rather than coliforms. Tests for this group are carried out employing similar media to those used to detect coliforms but with glucose substituted for lactose. Thus, detecting non-lactose or slow lactose fermenting strains of *E.coli* some of which may be pathogenic and the lactrose negative pathogenes e.g. *Salmonella* and *Shigella*.**



E.coli
(Non lactose fermenting strain)

RAPID METHODS

ATP Photometry

Luciferin+ luciferase+ ATP+O_2 ⟶ Luciferin+ luciferase+ AMP+ Light

Amount of ATP in microbial cells:

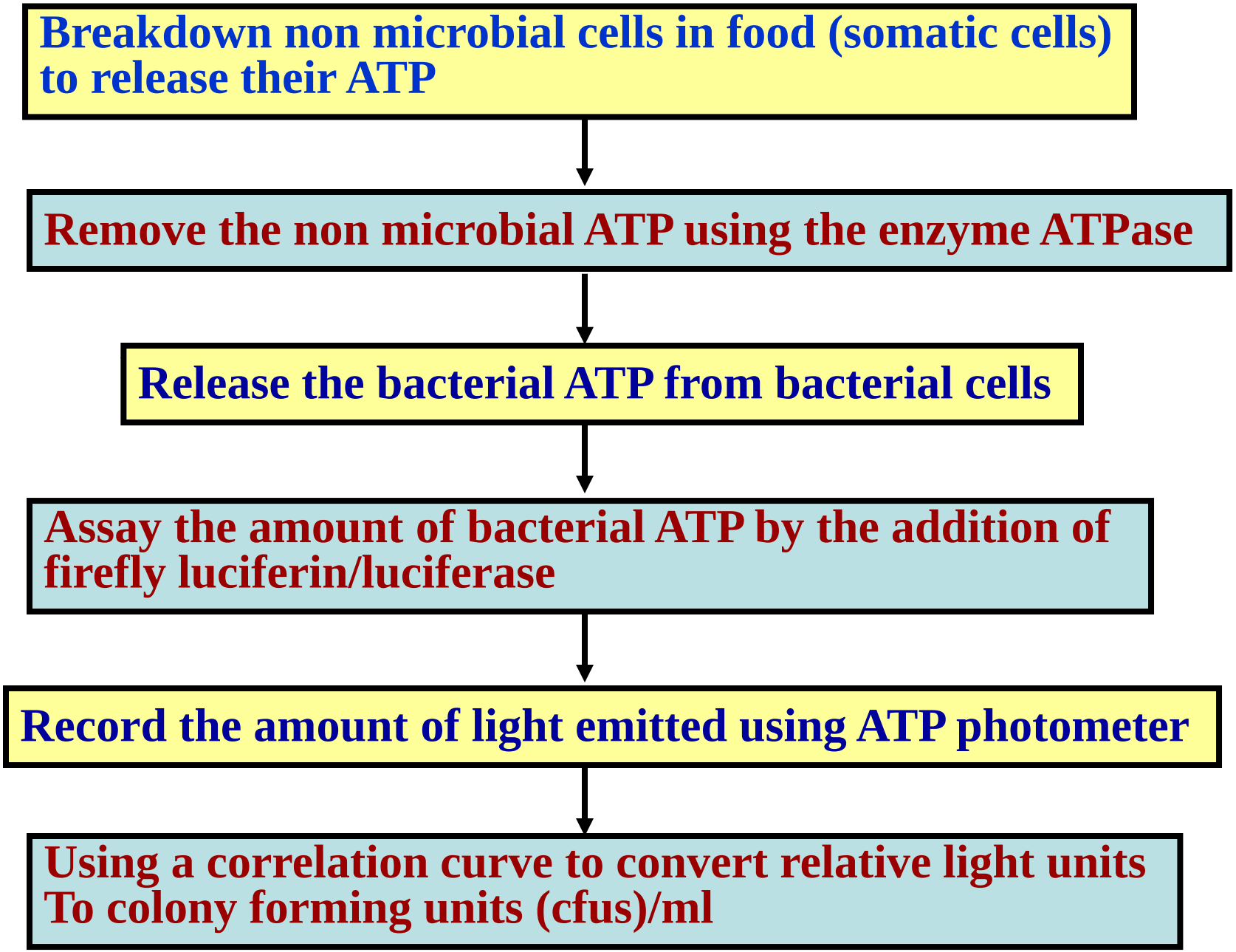
Bacterial cells contain~ 1 fg of ATP

Yeast cells contain~ 100 fg of ATP

- **ATP photometry has been used successfully to assess the quality of fresh meat and milk**
- **To measure the activity of starter cultures for sterility**
- **Technique is particularly useful for the rapid monitoring of surface contamination of processing equipment in the food industry**

fg : femtogram

Analysis of food using ATP photometry



Direct Epifluorescent Filter Techniques (DEFT)

- **DEFT was originally developed to monitor the microbiological quality of milk.**
- **DEFT uses a combination of direct microscopy and membrane filtration to assess the numbers of organism in food samples**
- **Food normally need some form of pre-treatment to enable sample to pass through the membrane filter**
- **The filter membrane needs to be pretreated using a surfactant to emulsify fat globules and proteolytic enzyme (trypsin) to remove somatic cells and allow the milk to pass through.**

Electrical impedance method

- **Impedance is the resistance to the flow of an alternating current through a conducting material e.g. microbiological culture medium.**
- **Microbial growth normally leads to decrease in impedance.**
- **The response is greatly influenced by the chemical composition of the medium and media are engineered to maximize the effect.**
- **Equipment used for monitoring the changes in impedance is bactometer.**

Immuno assay

- **Enzyme-linked immunosorbent assay (ELISA) technique takes only 90 minutes to complete.**
- **Pre-enrichment and enrichment stages similar to those used for the traditional analysis are required to increase cell numbers to a level that can be reliably detected (10^5/ml).**
- **Overall the gain is 2 days over the traditional method.**
- **ELISA has also been developed to test foods for bacterial toxins such as *Staphylococcus enterotoxin* and *mycotoxin.***

Flow cytometry

- An optically based method for analyzing individual cells in complex matrixes.
- It is used to estimate the number ,size and shape of microorganisms.
- Sensitivity of the technique is very high (10^2 yeast cells; 10^2 -10^3 bacterial cells per ml can be detected within few minutes.
- Suitable for detecting low numbers of specific organisms in fluid.
- Use to enumerate viruses in sea water.

MOLECULAR METHODS

Polymerase Chain Reaction (PCR)

Advantages of PCR

- The ability to utilize minute samples to produce a high yield of amplified target DNA.
- The specificity of the reaction
- The flexibility of the method.
- The simplicity and speed of the automated procedure.

Problems of PCR

- Inability to distinguish between live and dead cells
- The presence of polymerase inhibitors in food samples
- The accessibility of the target organisms

Pre enrichment of the test samples overcomes most of these problems and presently needed for detection of specific pathogens in food.

Immunofluorescence

- **Immunofluorescence is the labeling of antibodies or antigens with fluorescent dyes.**

- **Use of direct labeling (Primary Antibody directly labeled with flurophore).**

a) **Reduces the number of steps in the staining procedure.**

b) **Avoids cross-reactivity and high background problems.**

- **Fluorescent labeling can be performed in less than one hour with readily available labeling kits.**

Western blot

The western blot is a method of detecting specific proteins (Toxins) in a given sample of microbial contaminated food or extract.

Proteins from an microorganism (contaminated sample) are separated using electrophoresis

↓

The proteins are now transferred to a nitro cellulose filter by blotting

↓

The sample is incubated (3-4 hrs.) with the primary antibody over the nitro cellulose filter

↓

The sample is washed with the PBS buffer(3-4 times)

↓

Sample is incubated with secondary antibodies linked to Horse Radish Peroxidase (HRP)

↓

The sample is washed with the PBS buffer(3-4 times)

↓

Di-Amino Benzoic Acid (DAB, a substrate) specific to HRP enzyme will bind

↓

The band of interest will give a brown colour on nitrocellulose filter

DAB (substrate) + HRP (enzyme) ⟶ Brown colour band

CONCLUSIONS

1. It is important to realize that with any of the method of analysis for a pathogen or indicator, there is no absolute guarantee of success.

2. The organisms under test can be missed completely (false negative) or other organisms can mimic positive results giving rise to false positives.

3. Developing and improving methods of analysis for pathogens and indicators is an area of intensive and continuing research.

4. This is particularly the case where `new' pathogens are concerned and a widely accepted method needs to be established.

5 Even when techniques are well established, research continues to try and improve sensitivity, eliminate false positive and reduce the time taken to obtain results.

THANKS