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Practical Food Microbiology

3rd Stage

2nd Course

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Practical food Microbiology Syllabus 2020/2021

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Lab 1 Preparation of Samples Part 1

Food contamination: refers to the presence of harmful chemicals and microorganisms in food, which can cause consumer illness.

Food sampling: is a process used to check that a food is safe and that it does not contain harmful contaminants, or that it contains only permitted additives at acceptable levels, or that it contains the right levels of key components and its label announcements are correct, or to know the levels of nutrients present.

A. Sample collection

- **Identity of the food**
- Should contain common and alternative names
  - E.g. Maize, Beans, cowpea
  - Scientific name (Genus, species, variety)
  - E.g. *Zea mays*, *Vigna unguiculata*
- Plant food (entire plant/part e.g. roots)
- Animal food (entire animal/part)
- State of maturity (ripe or immature)
- Other details
- **Need to know**:

1. Number and size of sample to be collected
2. Distribution of samples
3. Stratification to be used
4. Sample label should be permanently attached to the sample
5. Common name of food
6. Sample code number
7. Date of receipt in Lab

- **During sample collection**:
- Collection details:
  - Date and time of collection
  - Name of collector
  - Place of origin
  - Sampling point/addresses (roadside stall, farm, market)
  - Condition of cultivation (feed regime)
  - Purchase price
  - Graphical record (Photograph, visual record with scale)
  - Transport conditions (mode and conditions of transport)

📖 **Description of sample collected after sample collection**

- Food type (Legume, fruit juice, milk product)
- Local use of foods (Famine, Festivals)
- State of food sample (solid, semisolid, viscous, or liquid)
- Process and preservation methods (canned or smoked)
- Preparation method (cooking)
- Extent of preparation (raw, fully cooked, reheated)
- Packing medium (brine, oil)
- Container or wrapping (can glass)
- Contact surface (can, glass)
- Label or list of ingredients (estimated by inspection)
- Batch number
- Weight of food collected/individual items
- Number of items
- Weight of common measure or portion

🔔 **Things to note**

1. Deliver samples to the laboratory promptly with the original conditions maintained as nearly as possible.
2. If products are in bulk: storage procedures, choice of containers, modes of transport should be considered.
3. Use containers that are clean, dry, leak-proof, wide- mouthed, sterile, and of a size suitable for samples of the product.

B. Sample Transportation

🔔 **Things to note**

1. Whenever possible, avoid glass containers, which may break.
2. For dry materials, use sterile metal boxes, cans, bags, or packets with suitable closures.
3. Identify each sample unit (defined later) with a properly marked strip of masking tape.
4. Transport frozen or refrigerated products in approved insulated containers of rigid construction.

Lab 2 Preparation of Samples Part 2

C. Sample Handling

🕮 During Handling

🔔 **Aim:** To protect the sample from changes in composition and contamination.

🔔 **Things to note**

1. Weight and nature of edible/inedible matter (Prior to further processing).
2. Method of preparation (Cooking or not, time, temperature of preparation).
3. Weight before/after cooking.
4. Ingredients added if any.
5. Method of mixing and reduction (grinding, homogenization)
6. Types of storage (addition of preservatives, temp of storage)
7. Methods used of take analytical samples
8. Storage of analytical samples or further processing
9. Name and signature of person completing record
10. Date of record
11. Other details

D. Sample Preparation

🕮 Preparation of analytical portions

- If the particle size or bulk is too large for analysis, it must be reduced in bulk or size for analysis.
- Documentation of sample preparation is very important.
- Separate edible/inedible portions, record descriptions and weigh all parts.
- Measure portion sizes, weights, volumes, density etc.

🕮 Receipt and storage:

🔔 **There are important notes about the food sample include:**

1. Size and nature of sample for examination: The quantity of sample submitted should normally be at least 100g.
2. Handling for examination: Contamination of the sample and microbial growth or death during transport and storage should be avoided.

🕮 Containers

- Instruments and containers that used in collection of food samples should be sterile.
- Samples taken from unpacked or opened cans or packets of food should first be placed in clean, dry and sterile leak-proof containers such as wide mouth glass of plastic jars with closures.
- The contain of sample should be secured with a tamper and labelled.
- **Information recorded on the label should include**:

1. Name of the food
2. Names of the sampling officers
3. Date and time of sampling
4. A unique classify identification number.
   - ➢ If the label is damage during transport the sample, so should be placed in a second container.

🕮 Sample Storage

- Keep ground samples in glass or plastic containers with air and water tight covers.
- Samples not analyzed immediately should be left in cold storage to minimize spoilage and other chemical reactions.
- Samples for lipid analysis – store under nitrogen at low temperature to prevent oxidation and unsaturated lipids.
- Light may initiate oxidation so store in dark containers.
- For lipid analysis, antioxidants may be added if they will not interfere with the analysis.
- It is therefore desirable to store a number of identical analytical samples.
- Minimize the number of staff involved in taking portions from them.
- **Samples should be transported and stored under conditions that inhibit changes in microbial numbers, so there are different ways to protect the samples until examination:**

- ➢ Frozen foods
- ➢ Chilled/iced foods (air temperature at or below 8Cº) and if possible, between (0Cº and 4Cº) but not frozen.
- ➢ Hot or warm samples and cooled down as quickly as possible to a temperature of 8 Cº or below.
- ➢ Dried foods and un-blown cans need not be cooled, stored and transported at a temperature less than 40 Cº.
- ➢ Insulated containers cooled by frozen ice or gel packs should be used to hold and transport chilled or frozen samples with volume at least 10% of the volume of the insulated container.

📖**Receipt and description at the laboratory**

🔔 **The following details should be recorded on the report form:**

1. Type of packaging: this may have an effect on the condition of the contents and should be recorded to aid explanation of the results.
   For example, the environment within vacuum packages is anaerobic.

2. Appearance describes the food sample in general terms.
   For example, 70g of machine-sliced, paper-wrapped, pink-colored, signs of deterioration, abnormal colour and mold should also be recorded.

3. Texture: Bacterial damage can cause products to become soft or semiliquid; this applies particularly to meat products.

4. Smell: this is an indication of spoilage (decay) or contamination. A full organoleptic test includes taste, but this should not be accepted in the laboratory.

Lab 3 Methods for Microbiological Examination of Foods

The reason for microbiological examination of foods is to identify the presence, types, and numbers of microorganisms and/ or their products (such as toxins) in food samples, which cause the spoilage of the food or pathogenic infections for the consumers.

🔔 **The microorganisms to look for:**

Indicator MO	Food poisoning MO	Infectious MO	Spoilage MO
• Coliform • *E. coli*	• Grow in food and cause diseases • Produce toxins and cause intoxication	• Food as vector not media	• Include fungi (yeasts and molds)

📖 **Microbiological examination methods**

1. Direct Methods

- Small sample volume & rapid technique.
- Inexpensive equipment.
- Total cell (living & dead cells)
- The examination of liquid & semi-solid Foods

A. Bright-field (light) microscopy:

i. Wet mounts.
ii. Hanging drop mounts (bacterial motility).
iii. Dry mounts (simple stain & differential stain).

B. Breed smears
C. Direct microscopic clump count (DMC) (Petroff- Hauser counting procedure).
D. Dark-field illumination and phase contrast microscopy.
E. Direct Epifluoresent Filter Technique (DEFT).

2. Indirect Methods:

A. Plate count
i. Standard Plate count
ii. Spiral Plate count

B. Membrane filtration
i. Suitable for **liquid or semi-liquid** samples (e.g., Water)
ii. Commonly used for **Coliform** and ***Staphylococcus* spp**.
iii. Useful for **small number** of M.O in sample (<25 CFU/ml)

C. Culturing Technique
🔔 **There are several different kinds of medium used:**

1. General media (Nutrient agar NA for bacteria and potato dextrose agar PDA for fungi)
2. Selective media
3. Differential media
4. Diagnostic media

D. Most Probable Number Method (MPN)
- Statistic approach to quantitate the numbers of bacteria, which utilize a multiple dilution to estimate the population of microorganisms in foods.
- Use to estimate the number of M.O (less accurate than the plate).
- Examine large amount of sample.
- The growth of M.O in the medium appear as **turbidity/change** in color of medium.
- Time consuming.

3. Alternative Methods
i. **Dye-reduction test**
- Estimation of viable microorganism that possess reducing capacities.
- More numbers of microorganisms less time for bacteria change the color of indicator dye.

- Use to determine raw milk quality.
- In this method two dyes are used, Methylene blue (blue) or Resazurin (pink).

ii. **ATP photometry (ATP bioluminescence)**

- This test based on detection of ATP in metabolically active cells through the production of light.
- The amount of ATP per cell is generally constant.
- Release fluorescence light depend on the amount of ATP in the food sample.
- And ATP measurement based on bioluminescence using luciferin-luciferase (substrate-enzyme) complex relies on oxidation of luciferin by enzyme luciferase.

$$\text{Luciferin} + \text{luciferase} + \text{ATP} + O2 \longrightarrow \text{Oxyluciferin} + \text{luciferase} + \text{AMP} + \text{light}$$

- The produced light, usually detected and quantified by sensitive luminometer device
- Rapid (1-2 min)

🔔 **Application**

- Microbial contamination of chicken, beef, milk and surfaces contamination.

🔔 **Disadvantage**

- Mixed bacteria and yeast cells.

iii. **Limulus lysate**

- The lysate protein is the most sensitive substance known for endotoxins.
- This test is performed by adding aliquots of food suspensions to small quantities of a lysate preparation, followed by incubation at 37ºC for 1 hour.
- The presence of endotoxins causes **gel formation** of the lysate material.
- Is a good and rapid indicator of the total numbers of **Gram-negative** bacteria.

🔔 **Application**

- Microbial spoilage of ground beef.
- Rapid evaluation of the hygienic quality of milk.
- Detection of Coliforms before and after pasteurization.

4. Rapid Method

A. Immunological Methods

- Enzyme-linked immunosorbent assay (ELISA).

B. DNA/RNA Methodology

- DNA hybridization.
- Polymerase Chain Reaction (PCR).

Lab 4 Microbiological Examination of Milk

The milk is an excellent medium for growth bacteria, yeasts and molds. The identification of microorganisms in milk and dairy products is very important for three principal reasons:

1. Pathogens or their toxins may constitute health hazards.
2. Spoilage microorganisms or their metabolites may cause spoilage.
3. Lactic acid of bacteria or other microorganisms may contribute to the preservation of milk and the production of desirable flavor and physical characteristics.

📖 **Normal flora of milk:**

- *Enterococcus faecalis*
- *Streptococcus lactus*
- *Lactobacillus sp.*
- *Candida albicans* (yogurt)

📖 **Pathogenic bacteria may present in milk**:

- *E. coli*
- *Streptococcus pyogenes*
- *Mycobacterium tuberculosis*
- *Salmonella sp.*
- *Brucella sp.*

🔔 **There are five major parameters that are routinely checked by regulatory agencies for quality raw milk production:**

1. Mastitis diagnosis through somatic cell counts and microbiological analyses.
2. Determination of microbial counts in bulk tank milk for verification of hygiene (cleaning, disinfection, cooling).
3. Testing for veterinary drugs and aflatoxins.
4. Spoilage (Determination of total viable counts of microorganisms).
5. Determination of psychrotrophic or thermotropic microorganisms.

📖 **Milk Examination methods:**

A. Microscopical Examination of milk

- **Procedure:** Put one drop of testing milk on the slide and examine it under microscope, then note the shape and type of microbial cells (sometimes stains must be used to view microbial cells).

B. Viable Bacterial Count:

- Prepare serial dilution of milk sample by delivering 1ml of milk to 9ml of **sterile** normal saline tube (10^{-1}), mix and transfer 1ml diluted milk from tube 1 to tube 2 (10^{-2}) continue until 5 dilutions.
- Prepare plates of nutrient agar media.
- Deliver 1ml of diluted milk 10^{-1},10^{-2} to Petri dishes and spread it using a spreader.
- Incubate the plates at 37°C for 24-48 hrs.
- Count No. of colonies and **multiply** by the dilution factor to determine No. of viable non pathogens in milk.
- A plate containing more than 300 colonies should not count, plate with more dilution is counted instead.

C. Dye- reduction test (Methylene Blue Reduction Test):

This is a rapid test to find the relative number of bacteria in a milk sample. The length of time for color change in a specific dye is proportional to the number of bacteria in the sample (more bacteria present, lead to faster reduction). On the other hand, the reductase test is based on the **oxidation-reduction** (O/R) activities of the viable bacteria present in milk. The short decolorization time refers to the high number of present bacteria in milk, and the poor quality.

Decolorization Time	The Quality Milk
30 min – 2 hrs	poor milk quality
2 – 6 hrs	fair milk quality
6 – 8 hrs	good milk quality
Over 8 hrs	excellent milk quality

D. Test for coliforms:

- Prepare test tubes contain MacConkey's broth.
- Add 0.1 ml of milk sample to MacConkey's broth.
- Incubate the tubes at 37 °C for 24- 48hr.
- Examine the tubes for the production of **acid** by **changing** the color of the medium from purple to yellow.

Lab 5 Microbiological Examination of Poultry, Meat, and Fish

The poultry, meat, and fish are rich with proteins, lipids, vitamins, and minerals. They also have good water content (moisture) with a suitable pH number (near the neutral). These all factors are suitable for growing and reproduction of most microorganisms.

The growth of microbes in meat, in general, is governed by a number of intrinsic and extrinsic factors. Intrinsic properties of meat, such as pH and moisture can promote microbial growth, whereas temperature is an extrinsic factor.

Fresh meat has a high-water content that is favorable for the growth of microorganisms. It also generally contains bacteria, including those that can cause diseases. The animals naturally carry bacterial species like *Salmonella* and *E. coli* in their intestines, and raw meat can become contaminated during the slaughter process.

Equipment and tools used in the processing of meat can also become contaminated with microbes and spread to the raw meat.

Bacteria multiply rapidly at temperatures from 4-60 °C. Pathogenic bacteria do not necessarily multiply in meat leading to illness. Some species such as *Staphylococcus aureus* tend to be outcompeted by other harmless flora or spoilage bacteria that lead to a bad odor that causes most consumers to discard the meat.

🕮 Beef

The most common pathogenic bacteria found in beef is ***Escherichia coli***. The *E. coli* strain O157: H7 is a rare, dangerous bacterium that can cause severe damage to the intestinal lining. ***Salmonella***, ***Staphylococcus aureus***, and ***Listeria monocytogenes*** are also common contaminants in beef. All of these organisms can be destroyed by cooking.

🕮 Chicken

Chicken is often contaminated with ***Salmonella enteritidis, S. aureus***, ***Campylobacter***, ***L. monocytogenes***, and ***E. coli*** can also be found in chicken. Chicken should be cooked to an internal temperature of **72-74 °C** to kill these microbes.

📖 **Fish** is faster than other meat products in spoilage because it has high moisture (high water content) which is suitable for the **enzymatic activity** of most microorganisms.
Bacteria mostly present in fish are belonging to the genus ***Vibrio***, ***Salmonella***, ***Shigella***, and ***Listeria***.
The source of bacteria in fish is the **contaminated water, transportation boxes, intestines and gills of fish or during cleaning**.

🔔 **Preventing Contamination in Different Kinds of Meat**

Food poisoning due to microbial contamination of meat can be prevented by **cooking the meat thoroughly** before consumption and observing **good hygiene practices** when cooking and handling meat. This includes the use of **clean utensils, cutting boards, knives, and prevention of cross-contamination between raw meat and ready-to-eat foods**. If there is a gap in safe handling practices, cooked meat may still become spoiled through cross-contamination.

The spores of some pathogenic bacteria, such as *Clostridium perfringens* are not easily destroyed during cooking. The heat of cooking can activate those spores to germinate and develop into mature bacteria if the food is kept at favorable temperature for a prolonged period.

Raw meat should be cooked thoroughly before consumption. Ready-to-eat cooked meat should be discarded if it has been at room temperature for more than four hours. Cooked and raw meat should be stored in a refrigerator.

It is very important to **wash hands before and after handling raw meats**. Also, it is useful to **place raw foods in a sealed container or plastic bags** to prevent meat juices from dripping on other food.

📖 **Methods of microbial examination for different kinds of meat and fish**

The microbiological examination of the food is performed for **two purposes**: to determine the **presence of pathogenic microorganisms or their hazardous by-products** such as toxins to ensure the safety of foods and **to enumerate total or indicator microbes** to verify products' quality and monitor effectiveness of hygienic processes.

🔔 **Below is a list of methods approved by food safety authorities to test the quality of meat and meat products**

- **Aerobic Plate Count/Total Viable Count (TVC)**
- ***Escherichia coli* O157:H7**
- **Shiga-toxin producing *Escherichia coli* (STEC)**
- ***Listeria monocytogenes***
- ***Salmonella***

📖 **Methods we can use in the lab:**

A. Estimation of surface contamination of meat:

By swab, take a smear from surface of meat or fish and put it in tube contain 5 ml of D.W. then mix well. Or measure about 5cm^2 using sterile template and sponge and place it in a sterile plastic bag contains D.W. **See photos below!**

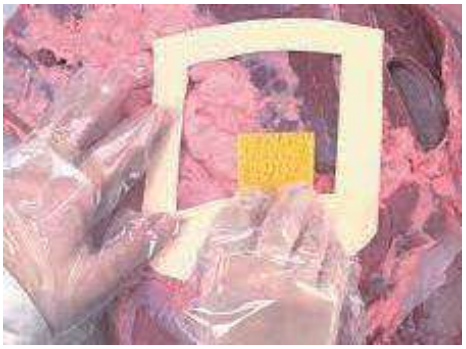
🔔 **Important things to keep in mind when applying this method in the field:**

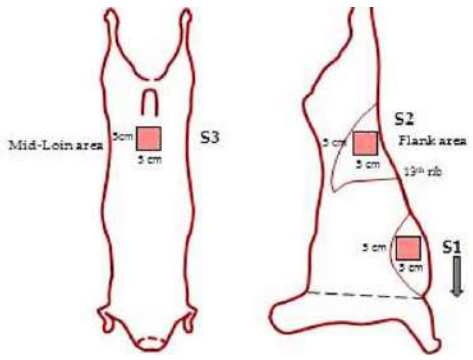
- Carcasses or surface swabs **must not be frozen** for transport. If a delay in transport of the sample is expected, the carcasses should be put aside and sampled at a time when the transport time/temperature objectives can be met

- Samples should be **dispatched on the day of collection** and **analysis** commenced on the **day following collection** and no later than on the second day following collection

- Bags containing sample swabs should be **firmly secured** to prevent leakage

- **Samples of frozen meat** can be maintained frozen for up to **7-days after collection**. Frozen meat samples can be held frozen during transport or transported at 0- 5°C/0-7°C to allow thawing during transportation. Frozen samples **must not be re-frozen** once thawed or transported at 0-5°C /0-7°C.





For detailed procedure of sampling from fresh meat using carcasses or swab method see the video in the following link: https://www.youtube.com/watch?v=GQXAN2HGpS4&t=313s

Where to apply this method:

Microscopic exam:
Prepare a slide from swab water and stain by gram stain exam to determine gram positive and negative bacteria. **See gram staining in the video provided with this lecture!**

Total number of bacteria or Total Viable Count (TVC):

1. Transfer loop full from swab water and streak on surface media like nutrient agar and *Pseudomonas* agar, as well as plate count agar
2. Incubate the Petri dishes at 30-37 ° C for 48hrs
3. Compare growth on the three media
4. Then estimate the total number of bacteria

Mold and yeasts:
1. Prepare potato dextrose agar medium (**PDA**), adjusted the **pH to 4**
2. Sterilized the medium in autoclave
3. Pour the medium in Petri plates, left them to solidify
4. Transfer 0.5 ml of swab water to the plates and spreading it using a spreader
5. Incubate the Petri dish at **22-25 ºC for 3-5 days** and count the number of fungal cells
6. Examples of mold and yeasts that could be found are: ***Aspergillus*, *Penicillium*, and *Saccharomyces***

How to calculate CFUs obtained from swabbed samples or carcasses:

Counts must be reported in CFU/cm^2. Calculate the number of CFU/cm^2 area of meat surface as follows:

$$CFU/cm^2 = No.\,of\ colonies\ on\ plate \times \frac{Volume\ of\ diluent\ added}{Area\ sampled} \times Dilution\ Factor$$

Note: if your sample was minced meat, the CFUs can be counted per gram or square gram (CFU/g or CFU/g^2).

- **The dilution factor is used to adjust the results for dilutions undertaken during testing. Factors for 10-fold serial dilutions are as below:**

Number of Tenfold Serial Dilutions	Dilution Factor
0	1
1	10
2	100
3	1000
4	10,000

B. Enumeration the total number of M.O:

Enumeration the total number of molds, bacteria, yeasts, and coliforms **to know the kind of contamination in meat and meat products**.

1. Weight 11 gm of meat and put it in blender or stomacher with 99 ml of dilution solution 0.1% peptone
2. Mix for 2 min
3. Make serial dilutions until the dilution 10^{-5}
4. Then transfer of the suitable dilution and put in a Petri dish, then add the suitable medium

- **Apply the following tests:**

i. **Total number of bacteria:** Transfer 1ml of dilution to the Petri dish; then add plate count agar or nutrient agar. Incubate at 30-37 ºC for 48hrs and count the number of bacteria in 11 gm.

ii. **Total Coliforms:** Transfer 1 ml of dilution to the Petri dish, add violet red bile salt agar (VRB), and incubate at 37 ºC for 24hrs. Count the violet colonies and estimate the number of Coliforms in 11gm. **See videos provided!**

iii. **Mold and yeast**: Transfer 1 ml of dilution to the Petri dish, then add PDA medium, incubate at 22-25°C for 5 days and count the number of fungi in 11 gm of meat.

Lab 6 Mid Exam 1

Lab 7 Microbiological examination of Fruits and Vegetables

The microorganisms exist on the fruit and vegetables surface coming from soil, water, air, insects and others. These microorganisms include bacteria and mold that are capable of decomposing cellulose and pectin and inter into fruits and vegetables, then they will cause the spoilage of these crops.

Vegetables are spoiled faster than fruits due to high pH in vegetables, while the low pH in fruits encourages the growth of molds and yeasts. The important vegetables' bacteria, which cause spoilage are: *Bacillus*, *Pseudomonas*, and *Erwinia*. While the molds are: *Alternaria*, *Penicillium*, *Aspergillus*, and *Fusarium* spoil fruits such as oranges and apples, whereas the fruit contains high amount of sugar such as grapes are spoiled by yeasts.

🔔 **There is another group of food possesses characteristics allow some microorganism groups to grow, for example:**

- Food with high percentage of sugars is suitable medium just for **Osmophilic Microorganisms** (**microorganisms adapted to environments with high osmotic pressures**).
- Salty food is suitable medium just for **Halophilic Microorganisms.**
- Dried food is suitable medium just for a few Microorganisms that can grow in low aqueous level, which is called **Xerophilic Microorganisms.**

🕮 Microorganisms in dry fruits and vegetables

Dry fruits and vegetables contain variety of microbial groups. The source of contamination of some organisms from farm, which were not killed during the drying process because it is **spores forming** and **heat drying resistance**. Whereas other contamination comes from various sources such as: after drying, through trading, during storage and during operations of re-hydration.

The pathogenic Microorganisms, which are thrive in dried fruits and vegetables don't threat health because they cannot grow and reproduce in low level of humidity. After the re-dehydration process of fruit and vegetables, some Microorganisms can grow and reproduce (May including pathogenic microorganisms). Therefore, it is important to perform quality and quantity microbial tests on dried food to control the food quality.

🕮Methods:

1. **Dry fruit:** Place amount of 10 gm of dry fruit in a sterile beaker containing 90 ml of sterile D.W with gentle agitation. Leave the mixture for 30 minutes until rehydration.

A. **Total bacterial number estimation:** Amount of 1 ml and 0.1 ml of mixture transferred by pipette to duplicate Petri dishes and pour the culture media (nutrient agar). Then incubate them for 3 days (at 30 ºC). Then, count the colony and estimate the total bacterial number for each gram.

B. **Lactic acid bacteria:** Amount of 1 ml of mixture transferred by pipette to duplicate Petri dishes and pour the culture media (orange serum and agar). Then incubate them for 2 days (at 30 ºC). Then, examine the colonies by Catalase enzyme detecting.

C. **Mold and yeast:** Amount of 1 ml of mixture transferred by pipette to Petri dish and pours the culture media (Potato Dextrose Agar PDA). Then incubate them for 5 days (at 22 ºC). Then, count the colonies and estimate the total number for each gram. Then, count the colonies and estimate the total mold and yeast number for each gram.

2. **Dry vegetables:** Samples are prepared as in the last case

A. **Total bacterial number estimation:** Prepare and count as in the case of dry fruits.

B. **Spores' bacteria (Flat sour):** Boil the water that contains dry vegetables for 5 minutes, then the amount of the evaporated water must be added again as sterile D.W. 2ml of water that contain the Dry vegetables is transferred by pipette to Petri dishes and pour the culture media (Dxtrose/ triptone violet promecrisol agar). Then incubate them for2- 3 days (at 55 ºC). Then, count the colonies and estimate the spore's number for each gram.

Lab 8 Microbiological Examination of Table Eggs

🕮 Spoilage of eggs

The majority of newly-laid eggs are sterile internally; however, contamination can occur with organisms that are potentially pathogenic for humans, especially *Salmonella*.

🕮 The main sources of spoilage are:

1. Shells contaminated by:

a. Fecal matter of hen
b. Cracking the eggshell
c. Improper washing
d. Storage techniques and handling

2. Changes not caused by M.O.

a. Shrinkage shown by the size of the airspace of the eggs
b. Changes in the physical state of the egg's contents seen by **candling or breaking**
c. As the egg ages, egg white becomes thinner and watery and yolk membrane weakens

3. Microbial spoilage of eggs

Mostly caused by bacteria more than fungi. The bacterial spoilage usually caused by Gram-negative bacteria: *Pseudomonas*, *Proteus*, *Salmonella*, *Staphylococcus*, and coliforms.
Campylobacter and *Salmonella* are the most frequent pathogens associated with eggs. Less common are pathogens that include *Shigella* and *Listeria*.
The main risk of eating bad eggs is *Salmonella* infection, which can cause diarrhea, vomiting, and fever.
While infection with *Staphylococcus aureus* can be destroyed by cooking but their toxins are heat resistant. Staphylococcal symptoms include nausea, stomach cramps, vomiting or diarrhea.

a. Bacterial spoilage is called as rots. Consist of five types, green, colorless, black, pink and red rots.

1. **The green rots:** Caused by ***Pseudomonas fluorescens*** (grows at 0ºC). Yolk splits and blends with white.

2. **The colorless rots:** Caused by ***Pseudomonas*** and ***Acinetobacter*.** Detected by candling, yolk in later stages splits or shows a white covering.

3. **The black rots:** Caused by ***Pseudomonas*** and ***Proteus*. *Proteus melanovogenes*** causes black coloration yolk and dark color in white. This type of spoilage caused when eggs stored at temperature higher than the ordinary.

4. **The pink rots:** Less often, caused by ***Pseudomonas.*** Pinkish precipitation on the yolk and a pink color in the egg white.

5. **The red rots:** Most infrequently occurring one. Caused by a species of ***Serratia.***

b. Fungal spoilage of eggs: Spoilage of eggs by fungi goes through stages of mold growth. The stages give the defects their names. There are two stages:

1. Pin spot molding
2. Fungal Rotting

Some of molds that cause eggs spoilage are species of ***Mucor, Alternaria*** and ***Penicillium.***

🕮 **Testing the freshness of eggs using the egg sink or float test**

Use the egg float test to reveal if an egg is spoiled or bad. It works well when you use it with other factors such as looking for signs of spoilage, smells, etc.

The egg sink or float test is among the most accurate ways to test for an egg's freshness. That is because it relies on eggshell porosity and certain anatomical functions of the egg that directly influence its toughness, reflecting its age.

🔔 **What makes this process reliable has to do with how <u>eggshells</u> are structured.**

The shell of an average egg houses thousands of pores through which moisture, air, and microorganisms can pass through.

Before hatching its eggs, the hen coats their shells with a layer of film called **cuticle**, which **covers the pores and defends the eggs' contents** from spoilage-causing bacteria.

Fixed **between the egg white and shell are two membranes** that serve as additional **protective layers** against microbes.

These membranes bond closely to the shell right before an egg is laid, but **pull away from it as soon as it is laid**.

This then results in an **air space** forming between the layers. This **space will then function as a reservoir for oxygen for a chick** before it hatches.

As time passes from when an egg is laid, the layer of **protective film coating its shell withdraws, widening the pores and allowing more air to enter**.

At the same time, its oxygen reservoir also expands, providing more space for air to settle.

Both these events eventually lead to the egg **absorbing more air** and becoming more resistant, reinforcing the reliability behind the mechanics of the **float test in estimating an egg's age.**

🔔 When Eggs Float, What Does That Mean?

If eggs float when conducting an egg test, it tells you that the pores covering the eggshell are wide enough, giving it a toughness level that can only be achieved when an egg is old or "no longer fresh".

In fresh eggs, the pores are tinier, so there's little to no toughness, resulting in an egg that sinks to the bottom.

🖋 Candling test

The candling technique specifically tests for egg quality before selling. While it could also determine an egg's freshness, it's no longer as reliable when the egg tested is older.

For the candling method, place the candle next to that egg's large end to illuminate it. If you have an old or bad egg, the content doesn't fill the shell completely.

🖋 Testing Cracked Eggs

If you don't require the eggshell intact because you're about to cook the egg or use it in your recipe, there are several ways to check if it is still good.

1. Plate Test

The plate method is one of the simplest techniques. You need to crack the egg onto a plate to test its freshness.

If you see an orange or bright yellow yolk with a not-too-spread-out egg white, you have a fresh egg.

2. Sniff Test

If what comes out of the egg shell doesn't quite fit the description of a fresh or bad egg, give it a good sniff.

🕮 Storage of eggs

1. Refrigeration

- You'll want to store your eggs in a refrigerator with a temperature ranging from 4 to 7 degrees Celsius.
- Avoid storing eggs in your fridge's door since the fluctuating temperature from the opening and closing of this part can mess with their quality. Instead, place them in a carton and store them in the coolest section of your refrigerator.
- If you refrigerate them at the right temperature, eggs should have no problem lasting for eight weeks. Of course, they may no longer be fresh at that time, but they are still safe to consume.

2. Freezing

- If you want your eggs to last even longer than refrigerated ones, the best solution is to freeze them.

- To start, you need to break and beat them slightly. Then, grab a large ice cube tray and fill each cube with some beaten eggs.

- You may also separate the yolks from the whites, especially if you love cooking recipes requiring any of the two.

- This method should help keep your eggs for up to 12 months. **Take note that freezing eggs will reduce their freshness, which means it is best to use them for baking or an ingredient of any recipe.**

Determination of bacterial eggshell contamination

1. For the recovery of bacteria from the eggshell, the intact egg was placed in a plastic bag with 10 ml quarter-strength Ringer's solution (sodium chloride, potassium chloride, calcium chloride, and sodium lactate in the concentrations in which they occur in body fluids.) and the egg was rubbed through the bag for 1 minute.

2. The diluent was plated on Nutrient Agar for the determination of the total counts of aerobic bacteria and on Violet Red Bile Glucose Agar for the enumeration of Enterobacteriaceae. Plates were incubated respectively for 3 and 1 day(s) at 30°C.

Lab 9 Canned Food

🕮 **Canning is** a method of preserving food in permanent hermetically sealed and sterelized containers (metal, glass, thermo stable plastic or a multilayered flexible pouch). Many cans require opening by cutting the "end" open; others have removable covers. Cans may hold diverse contents such as foods, beverages, oil and chemicals, etc.

Canned foods are sterilized before being placed on the grocery shelf but if the sterilization has been unsuccessful, contamination or food spoilage may occur. Swollen cans most times do occur and usually contain gas produced by members of the genus ***Clostridium***.

🕮 **Food spoilage** is a process in which food deteriorates to the point in which it is not edible to humans or its quality of edibility becomes reduced. It therefore means that the original nutritional value, texture, and flavor of the food are damaged in such a way that the food becomes harmful to people and unsuitable to eat. Furthermore, **spoilage may be due to one or more of the following:**

- **Physical changes** such as those caused by freezing, burning, drying, and pressure.
- **Chemical reactions** between food and can's material or caused by catalyzing enzymes of microorganisms which occur because the sterilization was not enough to kill them, or the cans were not closed well that allow microorganisms to enter after sterilization.

🔔 **The important food groups:**

a. **Low acid food:** Meat, fish, poultry and dairy fall into a pH range of **5.0-6.8**. This large group is commonly referred to as the low acid group.
b. **Acid food:** With pH range values between **4.5** and **3.7**. Including fruits such as pear, oranges, apricots and tomatoes fall in this group.
c. **High acid food:** Such as pickled products and fermented foods. The pH values range from **3.7** down to **2.3** also jams and jellies are in this classification.

🕮 Types of Spoiling

A. Spoiling of canned food according to the condition and content of the can:

1. **Swell:** Bulging of both can ends by positive internal pressure due to gas generated by microbial or chemical activity. Either hard or soft swell.

2. **Flipper:** A can with normal appearance but one end flips out when the can is struck against a solid object but snaps back to the normal under light pressure.

3. **Springer:** Can bulge from one end which if forced back into normal position, the opposite end bulges.

4. **Leakage:** Due to perforated can or during insufficient sealing process.

5. **Overfilled can:** Has convex ends due to overfilling and not regarded as spoil.

B. Spoiling of canned food according to the cause:

i. **Microbial spoilage**: May result from insufficient sterilization processing or leakage.

ii. **Chemical spoilage**:

a- **Hydrogen swell**: Formation of hydrogen gas in can due to internal corrosion or scratch. Mainly occurring in acidic food (canned fruits). Quite harmless but undifferentiated from swell of spoiled can, so it is rejected.

b- **Sulphiding** (Sulphur stinker spoilage): Discoloration of can's inside with pink to dark purple. Occur due to reaction of sulphur-containing proteins (liver, kidney, tongue) with liberated H_2S from bacterial spoilage (*Clostridium nigrificans* {Sulphur stinker}) with the odor of rotted eggs. It may be accompanied with blackening when H_2S react with steel base of tin forming iron sulphide and may lead to pitting. Sulphiding can be prevented by sulphur resistant lacquer.

c- **Thermophilic anaerobic spoilage**: *Clostridium thermsccharolyticum* an obligate thermophile causes spoilage. The can swells and may burst due to production of CO_2 and H_2. The food becomes fermented sour, cheesy and develops butyric odor.

d- **Putrefactive anaerobic spoilage**: *Clostridium sporogenes* causes spoilage through putrefaction. The can swells and may burst. Putrefaction result from partial digestion of the food. The latter develops typical putrid odor.

iii. Rust and damage: Rust is reddish brown ferric oxide seen under label.

- Slight rust passes for rapid consumption.
- Sever rust condemned and rejected.
- Damage: Slight damage passes for rapid consumption. Whereas Sever damage rejected.

- **Flat souring**: High acid formation without gas production. Sour odor, bitter taste, container not swollen. It caused by thermophilic bacteria:

1- *Bacillus coagulans.*
2- *Bacillus stearothermophilus.*
3- *Bacillus circulans*. These bacteria attack CHO and producing acid without gas.

Lab 10 **Why you should avoid Canned food?**

- **Can <u>YOU</u> prevent health problems, cancer, disease, and illness through healthy foods, fitness, and a more relaxed lifestyle?**

1. **Bisphenol or BPA**

BPA is a toxic chemical that causes hormone imbalances and wide variety of health issues ranging from hypertension, aggression, obesity to cancer and heart disease.

2. **Imported Canned Food**

Imported Canned Food is even worse than American Canned Food.

- First, the foods are picked when they are not ripe and have 80% less nutrients than a fully ripe fruits and vegetables.

- Second, the facilities are not as hygienic and inspected on a regular basis as their counterparts in Europe and North America. Less than 2% of canned foods are inspected by FDA or Home Land security or any other organization.

3. **Leaking. Aluminum leaks**

In fact, most often foods are put into aluminum cans, then they will be sealed, and cooked, supposedly retain the freshness. Hence, it will certainly retain the aluminum free radicals hanging around after heating and contaminating the contents.

Over a period of time Aluminum accumulation in body can cause memory problem like Alzheimer's. Most canned foods like soups, vegetables, chicken or beef broth and tomato sauces are made of aluminum because it's more economical.

4. **Preservatives**

These preservatives are kept in state of non-compounding to other molecules. Extensive amount of sodium (salt) is used to keep the preservatives in canned

food from rotting. These preservatives are not harmful towards healthy people, but they may be harmful to pregnant women, babies, children, elderly, or anyone that is suffering from a chronic disease.

5. Low level food quality

If the quality of the ingredients is not that great or the fruits and vegetables look old and not so healthy, then they will be hidden from the eyes of supermarket shoppers and be forced into a can along with other such low-quality food, cooked up in a mass oven while still inside the can. Don't expect the ingredients inside your canned foods to be of high quality.

🔔 **So what should you do?**

Completely eliminate canned foods and if you are looking for your favorite tomato sauce use the ones in glass jars. Don't consume vegetables or grains in cans, simply buy fresh ones. The risk of developing many chronic diseases such as cancer, heart disease, obesity, diabetes, nervous system disorder and Alzheimer's goes down by consuming fresh foods that do not have any packaging.

Lab 11 Toxins (Poisons)

- **Toxic** refers to the condition of a substance and the degree to which it can cause damage to you or any other organism or system. In colloquial usage, toxic can refer to biological organisms and non-biological substances.
- **Poisonous** describes substances that will disturb organisms, usually in a harmful way. Poison implies a high level of toxicity, though any substance is technically poisonous if taken in a large enough dose. Poison always refers to biological organisms. So, in general, poisonous and toxic essentially refer to any substance or action that will cause you or anything else harm. These terms are often used interchangeably.

Types of poisons

In regard to poisoning, chemicals can be divided into four broad groups: agricultural and industrial chemicals, drugs and health care products, biological poisons (plant and animal sources) and radiation.

1- Agricultural and industrial chemicals:

The majority of agricultural chemicals are pesticides, which include insecticides, herbicides, fungicides, fumigants, and rodenticides. Whereas the term industrial chemicals are used to refer to chemicals used neither in agriculture nor as drugs. Therefore, it includes chemicals used in industry, as well as chemicals found in or near households. Poisoning with industrial chemicals occurs most often by either percutaneous or inhalation routes.

2- **Drugs and health care products:**

Poisoning with drugs predominantly involves oral exposures. With drugs, therefore, irritation of the respiratory tract is rare, but anorexia, nausea, and vomiting resulting from gastrointestinal irritation are common.

3- **Poisons of biological origin**

Biotoxins can be grouped into three major categories:

a. **Microbial toxins**, poisons produced by bacteria, blue-green algae, and golden-brown algae.

b. **Phytotoxins**, poisons produced by plants. Poisonous plants may be classified due to their toxic effects: (1) plants that are poisonous to eat, (2) plants that are poisonous upon contact, (3) plants that produce photosensitization, and (4) plants that produce airborne allergies

c. **Zootoxins**, poisons produced by animals. Poisonous animals are widely distributed throughout the animal kingdom; the only major group that seems to be exempt is the birds.

4- **Radiation,** Radiation is a flow of energy through space or matter. It takes the form of particles (*e.g.*, alpha and beta particles) or electromagnetic waves (*e.g.*, X rays, gamma rays, and visible and ultraviolet [UV] light). Radiation can be classified as either ionizing or nonionizing depending on its ability to produce ions in the matter it interacts with. Ionizing radiation is more toxic than nonionizing radiation.

Lab 12 Mid Exam 2