Ministry of Higher Education
and Scientific Research
University of Baghdad
College of Science
Department of Biology

Practical Food Microbiology
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المرحلة الرابعة – الدراستين الصباحية والمسائية

الفصل الدراسي الاول

تدريسي المادة:

أ.م. ديمة نزار م.د. نسرين هادي

م.م.علي محسن علي أ.م خمائل لطفي

د. مختار جواد كاظم

Lab 1 :Introduction

Food

is considered as a **good environment** for growth of many M.Os. **(Why?)**

- M.Os. cause **spoilage** that lead to large economical loss **especially** if we do not follow the correct method in marketing & storing.
- Food also considered as a **Carrier Media** for many pathogenic M.Os. which **cause diseases**, **(foodborne diseases)** such as:

Bacillus anthracis	Anthrax
Brucella melitensis	Malta fever
Vibrio cholerae	Cholera
Salmonella enterica	Typhoid disease
Mycobacterium tuberculosis	T.B.

Or cause **Food poisoning**, such as: **Bacteria**:
Staphylococcus aureus, *Clostridium perfringens*
Fungi : aflatoxin poison produced by *Aspergillus flavus*

The importance of food microorganisms come from:

- Prevent food contamination by these dangerous M.Os.

Causes of Food Contamination
Microbial Growth
Insects, Rodents & Birds
Physical Changes of food (Cooling, Drying)
Enzymes Activity normally found in foods

Sources of Food Contamination
Air
Water
Soil
Fertilizers (Compost)
Insects (disease carriers)
Food Handlers

how to collect the food sample?

The food sample must be:

1- Representative for the whole food material.
2- Randomly taken.
3- Taken under sterile conditions to prevent contamination or adding more M.Os.
4- Reserved in the same physical condition (frozen remain frozen, dried remain dried) **why?**
5- Transferred to the lab directly for further analysis.

Types of Food Samples

1. **Liquid Samples**
   ex: Milk, Juice, etc. Shake before sampling for homogenization.
2. **Solid Samples**
   ex: Fruits, Vegetables, etc. Sampling done by using a sterile knife or cork borer.
3. **Surface Samples**
   Sampling done by Taking thin layers from the surface of food sample.
4. **Anaerobic Samples**
   The sample should be deeply taken in the absence of air as much as possible & use appropriate diluted solution.

Carrier:

Is a tool or a method used to transport samples from food materials to culture media for the:

1) Protection of the resident M.Os in food materials without losing it.
2) Prevent contamination with another M.Os.

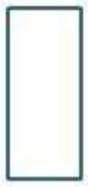
Types of Carriers:

1) **Replica**: direct method by pressing the food on the culture media.
2) **Rinse & Washing**: make stock solution from the food sample by rinsing & washing it in sterile diluent.
3) **Adhesive Tape:** two-sided tape; paper side to write the sampling details & sticky side to be pressed on the food surface & then on the culture medium.
4) **Agar Sausage**: solidified agar in a plastic cylinder, multiple agar culture can be cut & pressed on food sample.
5) **Contact Slide (Surface Slide)**: glass slide pressed on the food sample & then examined microscopically or pressed on medium.
6) **Swabs**
   a) **Cotton Wool Swab**: non-absorbent cotton wool rolled on wooden sticks & sterilized, directly rubbed on food then streaked on the medium.
   b) **Alginate Swab**: made from **calcium alginate** suspended in **1% calgon (sodium hexameta phosphate)**.

Types of Diluent Solutions:

1) **0.1% Pepton Water (pH=7):** protein samples.
2) **Phosphate Buffer**: water & dairy products.
3) **Sterile Distilled Water**: If there is no another diluent solution.
4) **Anaerobic Bacteria**: the diluent solution & the agar medium must be the same contents, ex: sulphid broth (diluent) cultured on sulphid medium.
5) **Osmophilic M.Os.** 15-20% sugar solution.
6) **Halophilic M.Os.:** 15-20% NaCl solution.

Plastic Basket contains the followings:



Alcoholic Burner	Lighter	Marker	Loop	Micropipette	Petri Dishes	Slide

1. **Clean the Bench using Detergent & Sponge.**

2. **Prepare The Food Sample Diluent.**

- **Low Contaminated Sample**
- **(1st. – 2nd. Dilution) Why?**
- **Highly Contaminated Sample**
- **(Serial Dilutions … 10^{-x}) Why?**

5g from Food Sample	45ml of Distilled Water	1st. Dilution from Food sample

3. **Light the Burner**

4. **Mark the Petri Dishes**

Pouring Plate Method

1) Take 0.1ml from the food sample diluent using Micropipette.
2) Place the inoculum in the Center of the Petri dish.
3) Get rid of the Tip of the micropipette by placing it into the bin.
4) Pour the Cooled Medium on the inoculum & homogenize the inoculum with the medium by mixing it clockwise & anticlockwise.

- **Incubate the inoculated Petri dishes in the incubator at**
  - **37°C for 18-24hrs. for bacterial isolation.**
  - **25-30°C for 2-3days for yeast isolation.**
  - **25-30°C for 5-7days for mold isolation.**
- **Record the results in a scientific report including:**
  - **The microbial count.**
  - **Types & species identified by microscopic & macroscopic examination.**

❖ How to Cool the Agar Medium??

Agar media are in the water bath to keep it in a liquid state

Waterbath	**Agar media in the waterbath.**	**Cool the agar media with tap water.**	**Check the temperature with your hand palm, keep cooling if it still hot.**

I/Bacterial Colonies: Small Colonies with the surface or within or under the agar.

Gram Stain for Bacteria

1- Put a small drop of water on the slide.

2- Take a touch by loopfull from **one colony** from the Petri dish & mix it softly with the drop of water on the slide.

3- Fix the smear by heat 45° over the burner flame **(not** through the flame) for 3 times.

4- Add drop from **Crystal Violet (1-1.5min)**.

5- Wash carefully with Tap water.

6- Add a drop of **Iodine** (Trapping agent) **(1min)**.

7- Add **Alcohol** (decolorizing agent) **(60sec)**.

8- Add **Safranin (1-1.5min)**.

9- Wash carefully with Tap water.

10- Dry the slide in the air at room temperature.

11- Find a clear field at 10X, 40X.

12- Move to the oil lenses (**100X**) after adding a **small drop** of oil on the slide.

II/Yeast Colonies: Small or Large, Colored, Shiny Colonies.

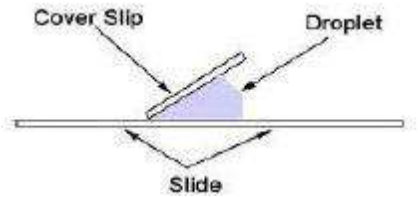
Simple Stain for Yeasts

1- Put a small drop of water on the slide.
2- Take a touch by loopfull from **one colony** from the Petri dish & mix it softly with the drop of water on the slide.
3- Fix the smear by heat 45° over the burner flame **(not** through the flame) for 3 times.
4- Add drop from **Crystal Violet (1-1.5min)**.
5- Wash carefully with Tap water.
6- Dry the slide in the air at room temperature, or at the hot air of the burner flame **not** through the flame.
7- Find a clear field at 10X. & Examine at 40X.

II/Mold Colonies: Large Colonies rise up over the agar.

Molds Slide Preparation

1- Place a drop of Lactophenol cotton blue on a slide.
2- Dig the mold colony from the agar by loop.
3- Put it over the slide constantly **without breaking it**.
4- Put a cover slide over it.
5- Knock carefully at the left angle to spread the colony under the slide cover **without breaking it**.
6- Find a clear field under 10X. & Examine under 40X.



Determination of M.Os Numbers:

) Total Count:

a) Breed Method
b) Haemocytometer

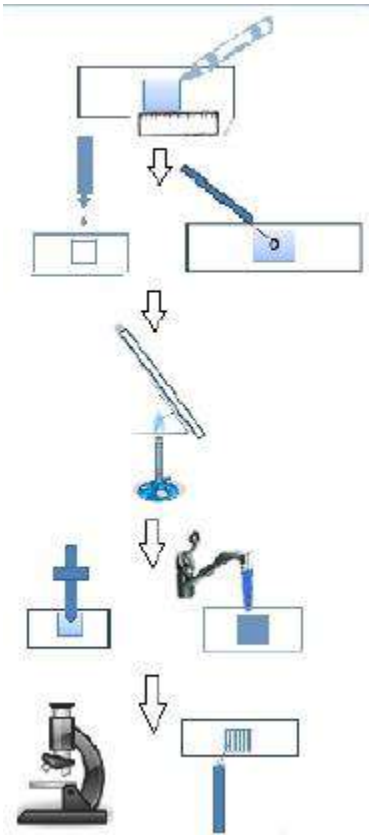
- Counts the dead cells, living cells & even food particles.
- Fast results within 10mintues or less.

2) Viable Count:

a) Pouring Plate Method.
b) Spreading.
c) Swabbing.
d) Most Probable Number (MPN).

- Count the living cells only.
- Results obtained within 24-48hrs.

Breed Method



1. Draw 1cm×1cm square on a clean slide with marker.

2. Flip the slide, put small drop of water on the slide & spread the inoculum by loop.

3. Fix the slide by 45° over the flame.

4. Stain the slide for 2min. Then wash with tap water.

5. Examine under microscope by counting the number of the stained particles in the examined field under oil lenses (repeat it for 10fields).

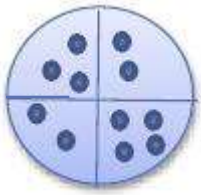
No. of cell in 1 field= #
No. of cells in 10 fields= #

Apply the formula below:

$$\text{Cell/ml} = \frac{\text{No. of bacteria in 10 fields}}{10} \times 100 \times 5000 \times 10$$

100= loopfull
5000=no. of fields in area for 1×1cm drawn square.
10=Inverse of dilution

Pouring Plate Method, Spreading & Swabbing



Count the colonies in the plate. Or in 1 quarter & multiply it by 4.

Apply the formula below:

CFU= No. of Colonies × Invert of dilution Factor × ?

? =

Inoculation factor= 10 (if the inoculum was 0.1)
Inoculation factor = 5 (if the inoculum was 0.2)
Inoculation factor= 2 (if the inoculum was 0.5)

To obtain the no. of cells in 1ml

TMC
(Too Much to Count)

Few colonies

Lab3: Bacterial Indicators of Food Contamination

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Health organizations

Concern about food free from pathogenic bacteria **because** of foodborne diseases. Danger come from vegetables watered & fertilized with sewage water. There are 3 bacterial groups found in human & animal feces that are considered as indicators for fecal contamination:

1) **Coliform.**
2) **Fecal Streptococci.**
3) **Gas producing Closteridia.**

I/Coliform (*E.coli*):

Gm-ve, coccobacilli, non-spore former, lactose fermenter, gas producer when grown at 37°C for 48 hrs., present in high numbers in human & warm blooded animals' feces, **detected by:**

1) Presumptive Test

- Inoculate **lactose broth** from the serial dilution of minced meat sample in **peptone water.**
- Incubate at 37°C for 48hrs. **+ve result:** Gas production (**bubble in Durham tube).**

2) Confirm Test

- Streaking **+ve result of presumptive test** on Endo agar or EMB (Eosin Methylene Blue).
- Incubate at 37°C for 48hrs. **+ve result:** Pink colonies on **Endo agar** & Green Metalic Sheen colonies on **EMB**.

3) Complement Test

- Inoculate **lactose broth** with the **+ve result of Confirm test.**
- Incubate at 37°C for 48hrs. **+ve result**: Gas production.
- For more confirm examine the cells under microscope.

Ejkman Test

Test done to detect the fecal bacteria by inoculating the **doubt samples** in lactose broth & incubating it at **44.5 °C**. Only fecal *E.coli* can grow in this temperature & ferment lactose to acid & gas.

II/Fecal Streptococci:

- Take Cheese & make **serial dilutions** with **Na-acetate** or Milk with **Peptone water.**

1) Presumptive Test

- Inoculate **azid dextrose broth** from the serial dilution. Incubate at 37˚C for 48hrs. **+ve result** – Conversion of broth to Yellow.

2) Confirm Test

- Transfer from the +ve tubes to **Ethyl Violet Azid broth**. Incubate at 37˚C for 24 hrs. **+ve result** Violet ring at the bottom of the tube or as heavy (extensive) turbidity.
- For more confirm examine the cells under microscope.

III/Gas producing Clostridia (*Clostridium perifringenes*):

- Colonize human & warm blooded-animals intestine (normal flora).
  - Its spores resist some thermal treatment.
  - The indication of these bacteria is uncommon, because of the **difficulty of cultivation**, but it is considered as a **complement test** for *E.coli* & *Streptococcus faecalis* tests.

1) Presumptive Test

- Take the food sample & make **serial dilutions**
- **Heat** the serial dilution at 80°C for 15 min (to kill the vegetative cells & survival the spores)
- Inoculate **milk broth** & then incubate at **37˚C for 5 days**. **+ve result** – Stormy Fermentation (High production of Acids & Gas).

2) Confirm Test

- Inoculate on selective medium **D.R.C.M (Differential reinforced Closteridial Media)** incubate at **45˚C for 24hrs**. **colonies** appear pink after adding NaOH for 20-30sec.
- Antibiotic containing media (Polymixm B & Cycloserine) can be used to prevent contamination with other bacterial species.

Lab4:Microorganisms in Red meat, Chicken, Fish &egg

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Meat

Containing **carbohydrates**, **nitrogen compounds**, **salts** & **minerals** beside elevated **moisture** & appropriate **pH;** make it **an excellent media for microbial growth & reproduction**, which may lead into unwanted changes.

- **Microbial Flora** are inside the **meat & on its** surface which come from many different sources.
- **Bacterial Count** of the healthy animal muscle tissue usually much lower than its surface **but it increases** when exposed surfaces become contaminated during & after slaughtering or butchering.
- **Bacterial Contamination** of meat is determined by :
  - **Rapid Examination,** Gram stain for a contact slide pressed on meat sample.
  - **Cultural Examination,** is done by taking thin superficial samples by sterile scalpel (طرشم) & forceps.
- **Cooking** will destroy the **Mesophilic** microflora of the raw meat, even **Thermoduric** bacteria ex.: *Closteridium perifringens*. But improper storage after cooking can increase the **Thermophilic** survivors.
- **Healthy Methods** in slaughtering, transporting, marketing & storage should be followed:
  a) **Physical Methods** Cooling, radiation.
  b) **Chemical Methods** by adding of preservatives (**Lactic** acid & **Acetic** acid).

Examples for microbial contaminants of meat

Bacteria		Molds
G-ve	G+ve	
Pseudomonas	Bacillus	Mucor
Salmonella	Lactobacillus	Rhizopus
	Leuconostoc	Sporotrichum
	Micrococcus	Cladosporium
	Staphylococcus	Penicillium
	Streptococcus	

I/Red Meat

A) Fresh Red Meat:

Sources of contamination include:

1) Soil, washing & drinking water, slaughter (bleeding, cutting up & handling).
2) The workers (hands & clothes).
3) Transporting & Marketing.

Types of microbial spoilage in fresh Red meat:

1) Off- odor & Sliminess:

Change of odder then forming slime materials on the surface of meat mainly by *Pseudomonas*.

2) Discoloration:

The appearance of **colored spots** on the surface of meat as a result of microbial growth:

Bacteria		Yeast		Molds	
Pseudomonas	**Green** spots	*Rhodotorula*	**Red-pinkish**	*Cladosporium*	**Black** spots
Serratia	**Red** spots			*Sporotrichum*	**White** spots
				Penicillium	**Green** spots

3) Putrefaction & Rancidity:

Protein in meat $\xrightarrow[\text{Protease producing M.Os.}\textit{Pseudomonas}]{\text{Putrefaction}}$ $NH_3 + H_2S$ + Putrefied compounds.

Lipid in meat $\xrightarrow[\text{Lipase producing M.Os.}\textit{Pseudomonas}]{\text{Rancidity}}$ Fatty Acids + Glycerol + Rancid odor

4) Meat Souring:

Occurs when meat is stored at room temperature:

Carbohydrates (CHO) in meat $\xrightarrow[\text{Mesophilic bacteria}]{\text{Oxidation by}}$ **Organic Acids**

(Coliform *Lactobacillus*)

B) Hash Meat:

High microbial contents, (Why?) from multiple sources of contamination:

1- The usage of hash **meat machines** that increase the exposed surface area.
2- Mixing the **contaminated parts** with **uncontaminated** ones.
3- Addition of contaminated **vegetable**, **grains** & **spices**.

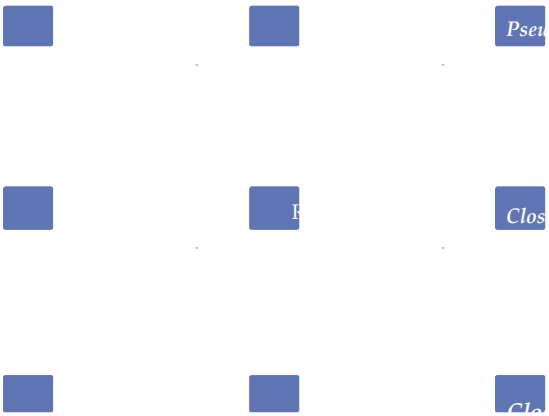
II/Fish Meat:

It is spoiled **faster** than red meat, because of:

1) High moisture.
2) High pH.
3) Lipids in fish oxidize faster than red meat.
4) The tissues` fish are softer & more disassemble(ككفم) than red meat.

- The microbial flora of fish is the same as the microbial flora of the water they come from.
- To preserve fish meat it should be:
  a) Cooled & kept in low temperature.
  b) Preserved by the addition of salts or acids to decrease pH.
  c) Clean from the supplying source.

Bacterial Flora of Fish



III/Chicken:

Chickens' environment is full of different kinds of M.Os. from many **contaminating sources** (field & its contents of drinking water, wastes & fodder [فلج]). So chicken must be cooked well. M.Os. of chickens include:

G+ve/ *Staphylococcus, Streptococcus, Clostridium, Lactobacillus.*

G-ve/ *E.coli, Pseudomonas, Salmonella*

properties prevent their spoilage, which include the followings:

1) **Physical Protection** from the solid calcic shell which prevents the entrance of M.Os. unless it is broken & contaminated with animals' feces or soil.
2) **Chemical Protection** include:

a) **Albumen** (egg white) which is not suitable for microbial spread, because of:

- Alkalinity of albumin (**pH=9.6**).
- It contains enzymes (**lysozyme**) that cause lyses of the cell wall of G+ve bacteria.
- Stickiness & gelatinous material (jellylike) will prevent the movement & spread of bacteria.

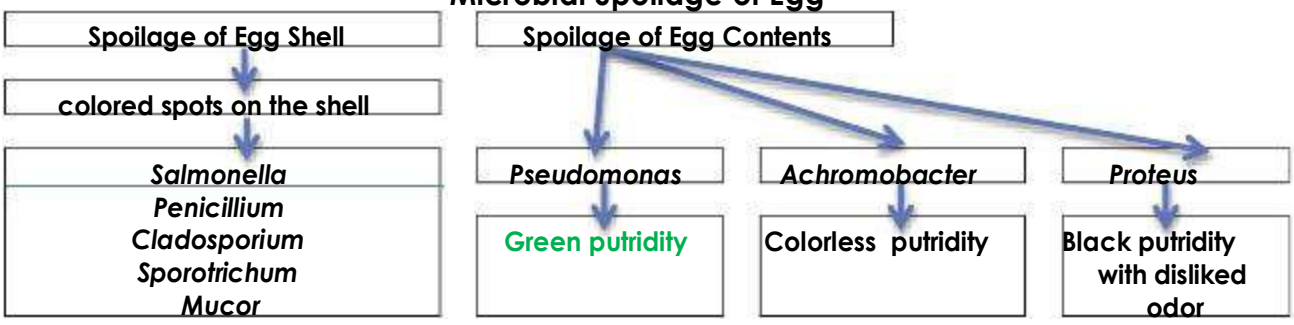
b) **Egg yolk**

A thin membrane surrounding the egg yolk will prevent the bacteria that can penetrate & cross the albumin.

IV/Eggs:

Perfect enriched media for microbial growth **(Why?)** (its contents of proteins, lipids & vitamins), **but** eggs have some special

Microbial Spoilage of Egg



To kill ***Salmonella*** & other bacteria that can spoil the eggs:

Pasteurization of the egg at 60°C for 2-3 min.

Washing the egg shell can decrease the No. of M.Os.

Laboratory Work:

A) General Examinations:

1) Compare the **odor & appearance** of the samples of different kinds of meat.
2) **Breed Method** for each sample to note the numbers & type of M.Os.

B) Extended Examinations

Pouring plate method for all samples as the followings:

1) Red Meat Sample

Nutrient Agar & Milk agar.

2) Hash (Minced) Meat Sample

Mannitol salt agar & MacConkey Agar

3) Fish Meat Sample

MRS or Rogosa & Staph 110 Agar

4) Chicken Meat Sample

S-S Agar & Nutrient Agar

5) Egg Sample

a) Content – Nutrient Agar.
b) Shell – Malt Agar & SS Agar

Lab5:Microorganisms in Fruits &Vegetables

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Microorganisms

Attach (infect) the crops of fruits & vegetables during the **growth** of the plant, **harvesting stages**, **storage**, **transport & marketing**.

Microbial spoilage in Fruits & Vegetables include:

I/Microbial Spoilage in harvesting Stages:

1) Pre-mature (before collection): **Bacteria** & **Molds** may cause spoilage, it depends on:

a) Suitable control.

b) Active mode of cultivation.

c) Fruits & veggies content like **acids** & **inhibitor materials** which inhibit microbial activity. The normal fruits & veggies internal components may still healthy if the outer layer (skin) was undamaged.

2) Post-mature (after collection): The degree of spoilage depends on the way of dealing with fruits from the harvesting stage to the consumption by consumers. If the outer layer is **scratched** or **damaged** the M.Os. can enter from water, air, soil, fertilizer. Some M.Os. can normally enter the fruits from the **natural pores** on its surface. The **chemical content** of the fruits **change after harvesting** as a result of **respiration** & **enzymes activity** which reduce acidity & inhibitors components causing microbial spread.

II/Microbial Spoilage from Chemical Nature:

The **pH range** & **sugar types** determine the nature & type of M.Os., causing the spoilage.

1. Fruits: **pH (2.5 -5)** , **molds & yeasts** are responsible for the spoilage & the source mostly the soil. They survive low pH beside high sugar concentration (65-70%) while **bacteria cannot.**

2. Vegetables: **Bacteria** are responsible for 36% of vegetables' spoilage because the **pH range is (4.5-7)** .

III/Microbial Spoilage according to Physical State:

1- Frozen Fruits: Molds & Yeasts cause spoilage because they can grow in:

- Low temperature.
- Low a_w under freezing.
- Absence of O_2 & CO_2.
- Ex.; **Yeasts**: *Candida, Rhodotorula* **Molds**: *Cladosporium,Botrytis*.

2- Dried Fruits Xerophilic molds & osmophilic yeasts cause its souring, because they grow in:

- Moisture less than **25%**.
- Temperature (**20-37C°**).
- Low a_w reach to 0.7.
- Ex.; yeasts: *Candida, Zygosaccharomyces*. Molds: *Aspergillus glaucus.*

The Most Important Spoilage Types on Fruits & Vegetables

Spoilage	Microbial Cause	Nature of Spoilage
Bacterial Soft Rot	*Erwinia carotovora*	- Lysis of pectin. - Watery soft figure with off-odder on vegetables
Souring & Slimness	*Pseudomonas*, coliforms, *Lactobacillus*	Vegetable Souring
Rhizopus Soft Rot	*Rhizopus*	Cottony growth with black spots & sliminess
Alternaria Rot	*Alternaria*	Black or Brown coloration
Gray Mold Rot	*Botrytis*	Gray spots on vegetables & fruits
Blue Mold Rot	*Penicillium*	Bluish-green coloration
Black Mold Rot	*Aspergillus niger*	Black growth

Laboratory Work:

1- Pouring Plate Method for all the samples on Nutrient Agar & Malt agar.

2- Microscopic Examination for the Results of the Previous Lab Samples.

3- Most Probable Number Method (MPN) for Green Vegetables.

MPN – Coliform counting method in samples contaminated with fecal source from sewage watering. Its formula:

Cell/ml=MPN value from the table × Invert of middle dilution Factor×?

To determine the MPN value we should follow the steps below:

1- We have 9 tubes from **MacConkey broth** divided into 3 sets, each set refer to a specific dilution 10^{-X}, 10^{-Y}, 10^{-Z}.

2- Inoculation of the tubes will be as below:

Calculations:

The conversion of the broth to yellow color refer to the positive result for **fecal coliform growth**, for example:

To calculate MPN number from the table we need to count the positive results as below:

Number of positive results in Set I = P1 in the table.
Number of positive results in Set II = P2 in the table.

Number of positive results in Set III = P3 in the table that include 5 columns.

MPN value calculated from matching these 3 results, for the results in the picture above:

MPN table value = 0.14 → Because P1=3 P2=2 P3=0

Cell/ml=MPN value from the table × Invert of middle dilution Factor×?

Cell/ml= 0.14 × 10^3 × ?

? =10 if inoculum was 0.1ml
or =5 if inoculum was 0.2ml,
or =2 if inoculum was 0.5ml

جدول (٤–١) قيم MPN لكل ٥ انابيب ملقحة لثلاث تخافيف متتالية من ١٠ تخافيف
ماخوذة من Mynell 1965

P_1	P_2	Most probable number for indicated values of P_3					
		0	1	2	3	4	5
0	0	-	0.018	0.036	0.054	0.072	0.090
0	1	0.018	0.036	0.055	0.073	0.091	0.11
0	2	0.037	0.055	0.074	0.092	0.11	0.13
0	3	0.056	0.074	0.093	0.11	0.13	0.15
0	4	0.075	0.094	0.11	0.13	0.15	0.17
0	5	0.094	0.11	0.13	0.15	0.17	0.19
1	0	0.020	0.040	0.060	0.080	0.10	0.12
1	1	0.040	0.061	0.081	0.10	0.12	0.14
1	2	0.061	0.082	0.10	0.12	0.15	0.17
1	3	0.083	0.10	0.13	0.15	0.17	0.19
1	4	0.11	0.13	0.15	0.17	0.19	0.22
1	5	0.13	0.15	0.17	0.19	0.22	0.24
2	0	0.045	0.068	0.091	0.12	0.14	0.16
2	1	0.068	0.092	0.12	0.14	0.17	0.19
2	2	0.093	0.12	0.14	0.17	0.19	0.22
2	3	0.12	0.14	0.17	0.20	0.22	0.25
2	4	0.15	0.17	0.20	0.23	0.25	0.28
2	5	0.17	0.20	0.23	0.26	0.29	0.32
3	0	0.078	0.11	0.13	0.16	0.20	0.23
3	1	0.11	0.14	0.17	0.20	0.23	0.27
3	2	0.14	0.17	0.20	0.24	0.27	0.31
3	3	0.17	0.21	0.24	0.28	0.31	0.35
3	4	0.21	0.24	0.28	0.32	0.36	0.40
3	5	0.25	0.29	0.32	0.37	0.41	0.45
4	0	0.13	0.17	0.21	0.25	0.30	0.36
4	1	0.17	0.21	0.26	0.31	0.36	0.42
4	2	0.22	0.26	0.32	0.38	0.44	0.50
4	3	0.27	0.33	0.39	0.45	0.52	0.59
4	4	0.34	0.40	0.47	0.54	0.62	0.69
4	5	0.41	0.48	0.56	0.64	0.72	0.81
5	0	0.23	0.31	0.43	0.58	0.76	0.95
5	1	0.33	0.46	0.64	0.84	1.1	1.3
5	2	0.49	0.70	0.95	1.2	1.5	1.8
5	3	0.79	1.1	1.4	1.8	2.1	2.5
5	4	1.3	1.7	2.2	2.8	3.5	4.5
5	5	2.4	3.5	5.4	9.2	16.0	-

Lab6: Microorganisms in Milk

Milk

Nutritional value to human beings from its rich content (proteins, carbohydrates, lipids, minerals, vitamins, pH (6.7) & optimal moisture) that can encourage the microbial growth leading to its quick spoil . **Un**pasteurized milk transfer some diseases, ex: Q-fever, Malta fever, & Food poisoning by Enterotoxins of *Streptococcus pyogens* .

Sources of Milk Contamination:

A) **Microbes during & after milking** (breast surface, soil, water, air, cattle feces, insects, flies & milk containers).

B) **Mechanical Milking**, the contamination ratio will decrease but all the used tools are an additional source of contamination especially when not cleaned or sterilized.

C) The **worker** is considered as an additional source for contamination.

Raw Milk

The fresh raw milk contains low number of bacteria but if its badly handled M.Os. can grow & spoil it quickly as below:-

1- Bactericidal Phase

Short Stage characterized by less no. of bacteria **(Why?), because** the raw milk contains antibacterial materials: **Lysozyme, Lactoferrins, Leucocytes & Lactenin** that is considered the most **effective**, it consists of 3 compounds (**Hydrogen peroxidase, Thiocyanates & Lactoperoxidase**) act together on bacteria.

2- *Streptococcus lactis* Stage

Activated in warm temperature **it ferment** the sugar milk (Lactose) quickly & produce lactic acid, until acidity reaches **1% the pH will decrease to 4.6**, that will stop its growth.

3- *Lactobacillus* Stage

It can resist more acidity & ferment the rest of Lactose to increase the acidity to 2% which will stop the growth of normal flora in milk.

4- Acid Oxidation Stage

After lactose conversion into lactic acid, acidity decreases by oxidation into H_2O & CO_2 will begin by mold & yeast: ***Geotrichum*** & **Membranous yeasts** (on the surface of the milk).

5- Putrefaction & Rancidity Stage

Bacillus, Proteus, Achromobacter & ***Pseudomonas*** will be active on the remaining lipids & proteins in the milk to convert them to putrefied & rancid liquid.

A) Raw Milk Spoilage:

Standard No. is 10^2 - 10^3 **bacteria/ml** in raw milk while it reaches 10^7 **cell/ml** in contaminated samples.

Types of Raw Milk Spoilage

Causative Agent	Spoilage	Type of Spoilage
Bacillus cereus	Coagulation	Production of Renin & precipitation of casein
Clostridium , Coliform	Gas production	Gassiness or frothiness in milk
Alcaligenes	Capsule production	Viscosity in milk
Ps. fluorescens	Fatty acid lysis	Undesirable taste (bitter taste)
Serratia marcescens	Pigment production	Red color in milk

B) Pasteurized Milk Spoilage:

- **Pasteurization** means: milk exposed to **72°C for 15 sec or 63°C for 30 min**, for prolong storage, & control pathogenic bacteria like ***M.tuberculosis, Salmonella, Brucella, Listeria***.
- **The resistance of** vegetative thermophiles ***Lactobacillus*** & ***B.subtilis*** cause its spoilage.

C) Dried Milk Spoilage:

Made by the removal of part of water in milk with homogenization process & heat treatment pre or post-canning takes place to prevent the spoilage. If the microbial examination of the dried milk showed positive growth for the viable count, then if it is:

- **Pure culture** means the contamination was by **thermophilic bacterial spores**.
- **Mixed culture** indicates that the contamination was caused from the **insufficient heat treatment** or happened **when following wrong procedure steps**.

D) Sterilized Milk:

Milk sterilized under 121°C for 15-20 min, packed in a glass bottle, paper-based, or metal bottle, in this manner all microbes will be killed. Spoilage may be related to the bad storage or caused by sterilization-heat resistant & spore forming bacteria, like ***Bacillus*** & ***Clostridium***.

Lab7:Microorganisms in Dairy

I/Cheeses:

Cheese is the hard product of milk. It is produced by the addition of lactic-acid bacteria as a starter or the addition of enzymes or acids followed by processes to give the texture & flavour of cheese.

Making Cheese

1- Treatment of Raw Milk

Sterilize the milk (Why?) to decrease the M.Os. that spoil cheese. The tempreture is different either **pasturazation or boiling**, it depends on the type of cheese.

2- Adding of Bacterial Starter or Rennete

Starter produce a sour flavour & precipitate the casien protien to make cheese

(Rennete): Is a raw extract from the four stomach of a calf. It contains an enzyme (Renine) that reacts with casein & make it precipitate.

3- Treating the Cheese Material

By **pressing** & **salting the cheese** to get it ripened.

- There are various kinds of cheese depend on the starter, tempreture or time of incubation & ripening method.
- **Ripening** : is a process in which cheese take the texture & flavour by using the enzymes (like Protease & Lipase) or by adding the bacteria & mold which are responsible for producing the type of cheese.

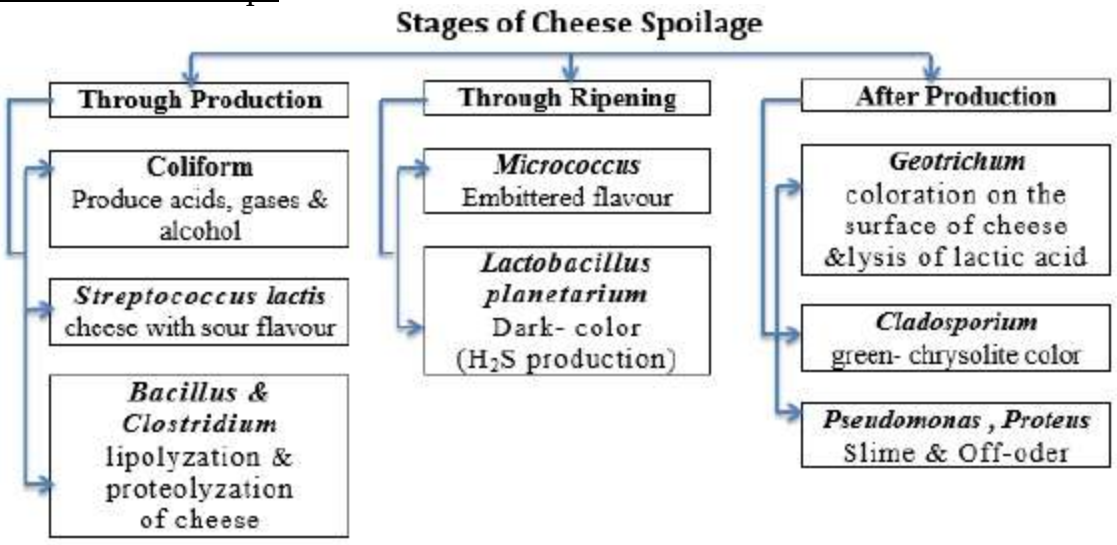
Spoilage of Cheese & Sources of Contamination

- **Spoilage of Cheese** depend on:

  a) Type of cheese. **b)** The moisture content. **c)** Tempreture. **d)** Period of storage.

- **Source of Contamination**

  It begins from the **raw milk**, heat treatment, the factory & it`s floor, tanks, water , bags, the handlers ,trucks , shops.



Listeria

1- Found in soil, water, food.

2- Speciese are non pathogenic & pathogenic like *Listeria monocytogenes* (**Gm+ve**, **pleomorphism** [may be shortbacilli , coccobacilli or curved like (V) letter in shape] **acrobatic motility** at **22°C** not 37°C for 18hrs. [survive in low tempreture]).

3- Cause **Listeriosis** includes: meningitis, abortion & dead infants in pregnant women, inflammation of animals udder, food poisining from contaminated food like milk, cheese, meat, vegetables because of it`s ability to produce heamolysin & enterotoxins that cause gastroenteritis.

4- Easily grow on culture media:

General Detection → Nutrient agar→**+ve** result-circular transparent colonies like (dew drops)
→Blood agar with sour odder or buttermilk like odder
→Trypton agar *It grows better in the presence of glucose in medium

Detection in Cheese → *Listeria* Enrichment Broth (L.E.B.) → Modified McBride Agar (M.M.A.)
→ +ve result shiny bluish-green colony

II/Fermented Milks (Yoghurt)

- **Fermented milk** is produced by the addition of *Lactobacillus bulgaricus* & *Streptococcus thermophilus* into fresh or dried milk after sterilizing & cooling then, incubated at **45-48°C** to produce curdling or thickening of the milk & to give it a typical sour flavour.
- **Sterilization** is very important to prevent the **contaminating bacteria** & **inhibit the enzymes**. Acidic flavour develops in yoghurt when increaseing the tempreture or the time of incubation.

III/Lipid Dairy product

A) Butter

- Made by the addition of a starter like *Streptococcus lactis* & *Streptococcus cremoris* into **sterilized milk** to produce lactic acid & decrease the pH to make ripened cream butter .
- Flavour of butter is made by adding a starter in addition of two kinds of M.Os. *Streptococcus citrovorus*& *Streptococcus paracitrovorus*, incubated at 22°C for 24hrs. then shaking in churns; the **floated butter drops are carried out, washed** sometimes salted to produce salted butter.

- **Spoilage of Butter**

  Less spoiled by M.Os. **(Why?)** as aresult of lipid content. Refrigeration & storage at low temperatures decrease microbial growth. But lipolytic, proteolytic M.Os. can grow at **low temperatures**, & cause discoloration, like *Geotrichum* as fungi; *Pseudomonas fluorescens*, *Pseudomonas fragi* & *Achromobacter* as bacteria which excreat (**lipase**) that produce short chain of fatty acids causing rancidity of butter.
- **Chemical spoilage** occur as aresult of production of short chain of fatty acids (like butyric acid) through **manufacturing** or **oxidation** & **lipolysation** of butter after manufacturing.

➢ **Margarin** is animals' or vegetables' oil inoculated by a starter of butter to smell like butter.

B)Cream

Sterilize the milk & cool it, the lipid layer will **appear** on the surface of the milk, its thickness depends on the lipid content of milk, this layer also contain quantity of protein , mineral salts , sugar of milk. The sterilized cream has a low microbial content, & the microbial spoilage may occur because of the M.Os. already presents in the original milk.

Labortory Work

1- Pouring Plate Method for All Diary Samples on Nutrient Agar.
2- Pouring Plate Method for Yoghurt Samples on Malt Agar.
3- Pouring Plate Method for Butter & Cream Samples on Oil Agar.
4- Milk Breed Method for all Diary Samples.

Lab8: Microorganisms in Bread & Cereal Grains

University of Baghdad/College of Science/Department of Biology /Food Microbiology LAB

Grains

Like rice & wheat are the most important sources in food consumption. Contamination begins from cultivation in the field either by: water, air, soil, insects, birds & rodents.

There are two factors control the **microbial growth & reproduction** in cereal grains:

- **Moisture.**
- **Storage Temperature.**

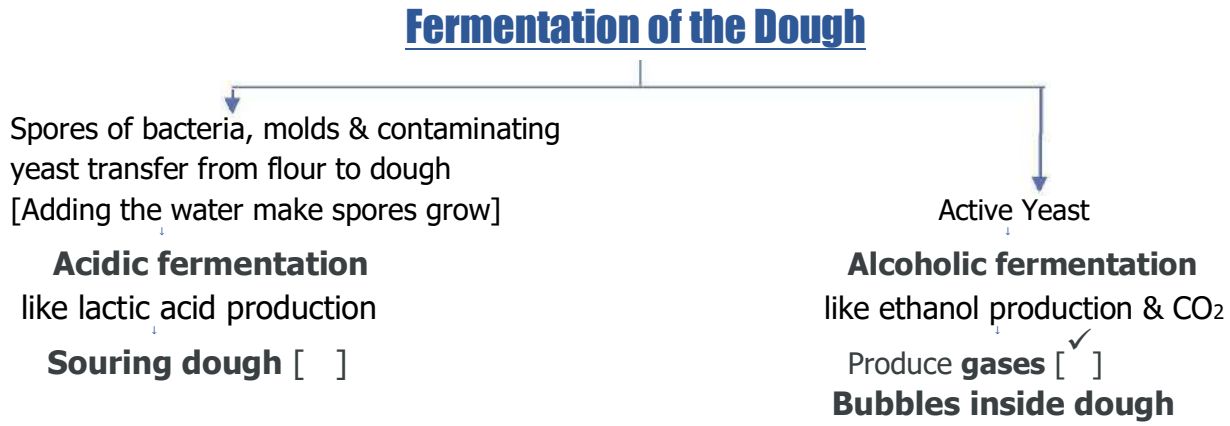
Cereal grains must be stored in a dry place (moisture <14%) **(Why?). Because** moisture encourage fungal growth especially those **toxin producers** such as: ***Aspergillus flavus***

Coliform count in flour & dough is advisable to detect such contamination despite the exposure for heat treatment which kill these M.Os.

Heat treatment may encourage the growth of ***Clostridium* & *Bacillus*** (***B. subtilis*** & ***B. mesentericus***) causing **bread ropiness** due to the production of capsular material.

Oven temperature kills all microbes present in bread dough expect heat resistant spores.

Bread is contaminated after baking from: tables, workers & insects beside the polyethylene sacs, which increase moisture so heat resistant spore growth may be encouraged.



Types of Bread Microbial Spoilage

A) Bread moldiness:

Happen due to molds growth on bread, ex.:

Mold	Type of Spoilage
Rhizopus	White growth spotted with black
Aspergillus niger	Black pins like growth
Monilia	Bloody bread (red-pinkish growth)
Mucor	White growth
Penicillium	Green growth

B) Bread Ropiness:

- ***Bacillus subtilis & Bacillus mesentericus,*** responsible for such spoilage because they are resistant to oven heat.
- Spores grow in bread & **produce ropiness & slimy materials** caused by gluten proteolysis & production of slimy peptides.
- These bacteria also **analyze the starch** into simplified sugars & undesirable organic acids which cause **Bread Acidity**

Laboratory Work:

1- **Pouring Plate Method for all samples on Nutrient Agar & Malt Agar.**
2- **2- Microscopic Examination for the Results of the Previous Lab Samples.**

Lab9:Microorganisms in Sugary Foods & Pickles

University of Baghdad/College of Science/Department of Biology /Food Microbiology LAB

I/ Sugary Foods

High sugar concentration are not suitable for the growth of many M.Os., therefore **Osmophilic** M.Os. **can play a major role in its contamination (Why?)** because they prefer high sugar concentration for their growth & reproduction.

A) Honey:

- Cannot be spoiled normally **(Why?)** because of its **sugar concentration** ~80%,
- **Spoilage can occur** when **humidity is elevated to 10% (Why?)** because of accumulation of water between sugar molecules (**Crystallization**).
- Honey may develop an **alcoholic yeasty flavor** when ethanol is produced **(Why?)** because of **fermentative reaction** which occur when **temperature is elevated** (**Yeasty Honey**).
- **Rapid spoilage** may occur when **crystallization increased** & **humidity ≥ 20%** especially in adulterated honey.
- **Pasteurization** for **30 min. at 60°C** must be done to preserve honey.
- **M.Os.** spoil honey include:
  Osmophillic yeasts: *Saccharomyces cerevisiae, Saccharomyces rouxii.*
  Molds like *Aspergillus, Penecillium & Mucor* on the surface absorbing humidity & O_2 from the atmosphere.

B) Debbis:

- Produced from dates, contain high percentage of sugar **(70-80-%)**.
- **Osmophillic yeast** (*Saccharomycees rouxii*) grow in 75% concentration of sugar & spoil the debbis **forming gases, alcohols & acids that change the taste**.

C) Jams & Candies:

Jams

- **Sugar** concentration **(70%)** but it doesn't prevent it from contamination **(Why?)** because they are **made from different kinds of fruits that may be a mixture of good & spoiled fruits**.
- **Heat** applied during jam's preparation might not be enough to kill all the spores or presented in the depth of spoiled fruit.

Candies & Chocolate

- **Rarely spoiled (Why?)** unless they're filled with contaminated stuffing or contaminated milk with spores of bacteria. In anaerobic conditions spores of *Clostridium* are activated forming gases that torn candies & their fillings goes out.
- **Contaminated nuts** with bacterial spores & fungal toxins are considered so dangerous.

II/Pickles

Made by **lactic acid fermentation** by **lactic acid bacteria**. Vegetables chapped into small pieces in **2-15% of NaCl**. **Acidity 1-1.5%** (Lactic Acid) gives flavor to the pickles & preserve it.

The Role of Lactic Acid Bacteria in Pickles

First Stage of Fermentation

(*Lactobacillus mesentroids*) has an important role of the fermentation in cabbage pickles, its growth increases until acidity reaches 0.1-1%.

Second Stage of Fermentation

Lactobacillus plantarium becomes more active **(why?)** because it tolerates acidity & can continue the production of lactic acid until it reaches the concentration of 2%.

Third stage of Fermentation

Lactobacillus brevis becomes active & change the remaining sugar into lactic acid reaching a rate of 2.4%.

In **olive pickles** the fermentation lasts for many months, in which *Lactobacillus plantarium* dominates on the last stage of fermentation; which also plays major role in the fermentation of **cucumber pickles**.

Pickles Spoilage

1- **Pickles Spoilage by Oxidative film yeasts**
Candida grow on pickles surface & oxidize the lactic acid to CO_2 & H_2O which form **a thin white film on pickles surface**.

2- **Pickles Spoilage by Fermentative Yeasts**
Torulopsis grow inside pickles producing large amounts of gases which make **pasteurization difficult** leading into **Floated Pickles**.

3- **Pickles Spoilage with Leuconostoc**
Forms a slime layer on the pickles producing **Slimy Pickles**.

4- **Pickles Spoilage with Bacillus subtilus**
It forms **Black Pickles** because it produces **H_2S** that reacts with the metal of cans forming a black residue of Fe_2SO_3.

5- **Pickles Spoilage by Molds**
Penicillium, Cladosporium that secretes pectinase enzyme that tears of the tissue of the pickles giving them soft appearance (**Soft Pickles**).

Laboratory Work:

1. Pouring Plate Method for Sugary Samples on Nutrient Agar +20% Sucrose & on Malt Agar.
2. Pouring Plate Method for Pickle Samples on Staph 110 & Malt agar & on Rogosa.
3. Microscopic Examination for the Results of the Previous Lab Samples.

Lab10:Microorganisms in Canned Food

Canning:

A process which is done either at home or for commercial purposes, it's steps summarized by putting the food inside cans, then sealed to be exposed to heat **(why?)** in order to store for a long period of time without spoilage.

Steps of Canning:

1- Preparation of the Raw Food

It **must be**:

a) Low contaminated.

b) Good quality.

c) Removing damaged parts.

2- Blanching

Prepare (vegetables or fruits) for freezing or further cooking by immersing briefly in boiling water. It is done **in order to**:

a) Reduce the microbial contents.

b) Stop the enzymatic activity.

c) Expulsion of air.

d) Reduce the size.

3- Filling

The cans must be filled without leaving a huge vacuum **(Why?)** in order to prevent the aerobic conditions for the microbial growth & oxidation stress.

4- Deflation (Exhausting)

Before sealing the cans, they must be heated in a water bath or steamed **(Why?)** to expel the air to prevent microbial growth & oxidative stress.

5- Sealing Dual Welding

must be applied **(Why?)** to prevent the formation of holes that would permit the entrance of the air or cooling water.

6- Thermal Processing

It is done to eliminate microbes & inhibit the action of enzymes, skipping this step leads to the damage of food. The **degree of heat depends** on a number of factors especially the **pH of food**. The foods with **neutral** acidity & **neutral pH** should be sterilized at 115-121°C for half an hour, while **acidic foods** are sterilized at 100°C for 20-30min.

7- Cooling

Treated cooling water (in order not to add contamination) applied directly after heat treatment **(Thermal Cold Shock) (Why?)** to prevent the thermophilic bacteria that resisted the heat treatment to grow.

Examination of Canned Foods

I/Physical Examination

1- Record all the information on canned food (trade mark, date of production & expiry).
2- Remove the trade mark then notice that if there were signs of oxidation, scratch, blemish or wrinkle on the can.
3- Notice if the can was flat or swollen & whether strong or weak swelling.
4- Wash the can with soap & water then expose the flat side (not the swollen side) to the flame.
5- Check the gas & its type by a special device to examine the **bulging cans**.
6- Empty the contents of the cans & check it to make sure there is no oxidation.

II/ Microbial Examination

A)Unspoiled Canned Foods:

Its applied to ensure the effectiveness of sterilization & the possibility of preserving the canned food, it include several stages:

1- Examining the Effectiveness of Sterilization

Open the canned food sample under sterilized conditions. **Use** sterile pipette **for Liquid** sample & sterile knife or cork borer **for solid foods** then **dilute** & **inoculate** on the suitable culture media depending on the type of food as the followings:

a) Canned Foods of Low & Moderate Acidity (pH≥4.5)

Inoculate Plato count broth or Litmus Milk broth **(Why?)** to detect the **aerobic** microbes which is then incubated at 30-32 °C. While we use Thioglycolate broth in detecting the **anaerobic** microbes. It is inoculated, then a layer over of Agar must be added **(Why?)** and incubated at a 32°C & 55°C.

b)Canned Foods of High Acidity (pH≤4.5)

Inoculate Orange serum broth & incubate at 30-32°C for the **detection of aerobic M.Os**, While detecting **anaerobic M.Os** is done by using the Orange serum broth then a layer of Agar must be added **(Why?)** & incubate at 30-32°C.

2- Examining the Stability of Canned Foods

The low acid canned food **pH < 4.5** incubated for a period of 7-30days & in different temperature degrees. While the food with **pH > 4.5** must be **incubated** it for 14days at 37°C & examine the boxes showing signs of corruption or an external swelling.

B) Spoiled Canned Food:

The **Microbial spoilage** occurs in cans because of the growth of microbes that survived the thermal treatment **(Why?)** either because of the inaccuracies in the treatment or a defect in the packaging that would permit the entrance of microbes after a thermal treatment; beside the chemical damage that may take place according to the interactions between food & metal enclosure or between the components of the food itself.

The Important Types of Spoilage in Canned Foods:

A) Spoilage caused by Spore former Thermophilic Bacteria

These bacteria can cause:

1- Flat Sour Spoilage

Bacillus stearothermophilus cause this spoilage, forming acids, mainly **Lactic Acid without Gas**, so the can remains flat & does not swallow **(Why?)** but when its opened it shows sour like odor (**ex:** canned vegetables, powdered milk & the milk conglomerates). This type of **spoilage happens** when the canned foods stored in the heat, besides the existence of the spores of these bacteria in food. **Dextrose trypton bromocresol purple agar** is used for the detection of these bacteria which must be then incubated at **55°C for 2-5days**.

2- Thermophilic Anaerobic Spoilage

Caused by ***Clostridium thermosaccharolyticum***, also called the **Gassy Spoilage (Why?)** according to the formation of large amount of gases.

3- Sulfite Spoilage

Caused by ***Clostridium nigrificans*** , specific serial dilutions from the sample then inoculating **Sulfur broth**, & adding **3% of agar (Why?)** Incubation at 55°C for 2-3 days, the **Black colonies** considered a positive result.

4- Proteolytic Anaerobic Bacteria

It is caused by ***Clostridium botulinum*** (**Putrefactive Anaerobic**), **Thioglycolate** medium must be used to isolate the bacteria & then incubated at **37°C**.

5- Spore-former Bacteria

Such as ***Bacillus subtilis***.

B) Spoilage caused by Mesophilic Non Spore-former Bacteria, Fungi & Yeast

Their presence **indicate** the inaccuracies of thermal treatment or contamination after thermal treatment, such as ***Lactobacillus***, ***Leuconostoc***, Staphylococci, Streptococci, yeasts in canned sweets foods

III/ Chemical Examination of the Canned Food

Chemical Reactions that happen between the food content & the can metal which lead to the production of H_2 or CO_2 or chemical reactions caused by ***Bacillus coagulans***.