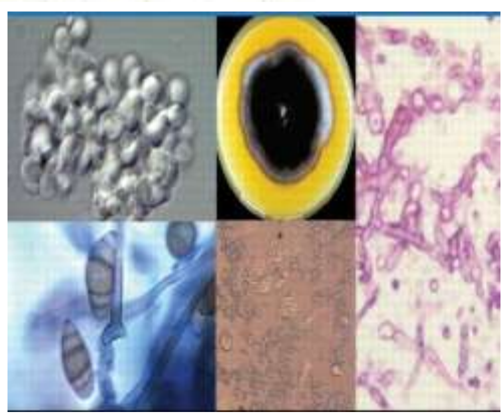
Kingdom of Saudi Arabia
Ministry of Education
Northern Border University
Faculty of Science and Arts
Department of Biology
(Microbiology)

Practical Food Microbiology Course

(3303-425)



Prepared by

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Lab (1): Food Spoilage

- **Definition of Food spoilage:**

A disagreeable change in a food's normal state, such as changes can be detected by smell, taste, or touch. After these changes the food becomes harmful to human and unsuitable to eat.

- **Causes of Food Spoilage:**

The changes occur in foods are due to a number of factors that are:

(1) **Physical factors:** such as changes occurs by temperature, air, moisture and light.

(2) **Chemical factors:** such as oxidation occurs for some compounds in foods through reactions with atmospheric oxygen.

(3) **Biological factors:** such as occurs by microbial growth, insect damage and some animals.

- **Signs of Food Spoilage:**

  - Changes in color.
  - Changes in odor.
  - Changes in texture.
  - Accumulation of gas or foam.
  - Accumulation of liquid.

- **Basic Types of Food Spoilage Signs:**

There are 3 basic signs of food spoilage that are: appearance, textural and flavor (smell and taste).

(1) Appearance: when a food “looks bad,” it may due to:

(A) Microbial growth it causes either:

<table>
<tr><td colspan="2">(1) Mycelia or colonies visible on surface.</td></tr>
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<tr><td colspan="2">(2) Development of cloudiness in liquids</td></tr>
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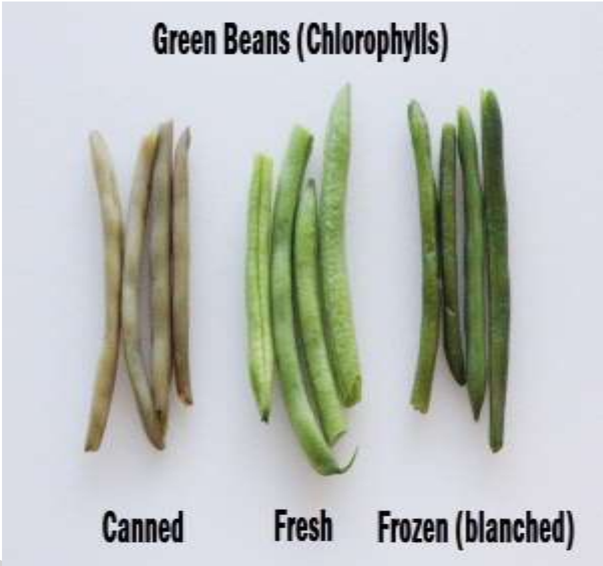
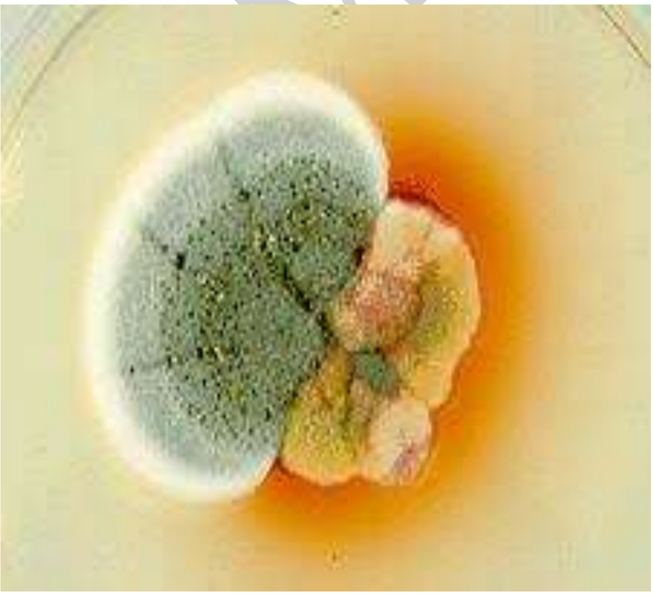
(B) Changes in food color which due to:

(1) Heme or chlorophyll breakdown.



(2) Pigments from microbial colonies and mycelia



(2) Textural changes (feel)

(A) Slime formation: due primarily to surface accumulation of microbial cells, also be a manifestation of tissue degradation

(B) Tissue softening: due to enzymatic degradation (e.g. soft rot in vegetables)

(3) Changes in flavor (taste and odor) which may due to formation of:

- Nitrogenous compounds (ammonia, amines, etc.).
- Organic acids.
- Sulfides.

	
(1) Change in taste (Organic acids)	(2) Change in smell (Ammonia or Sulfide)

Lab (2): Types of Food Spoilage

There are 3 main types of food spoilage that are (physical, chemical and microbial).

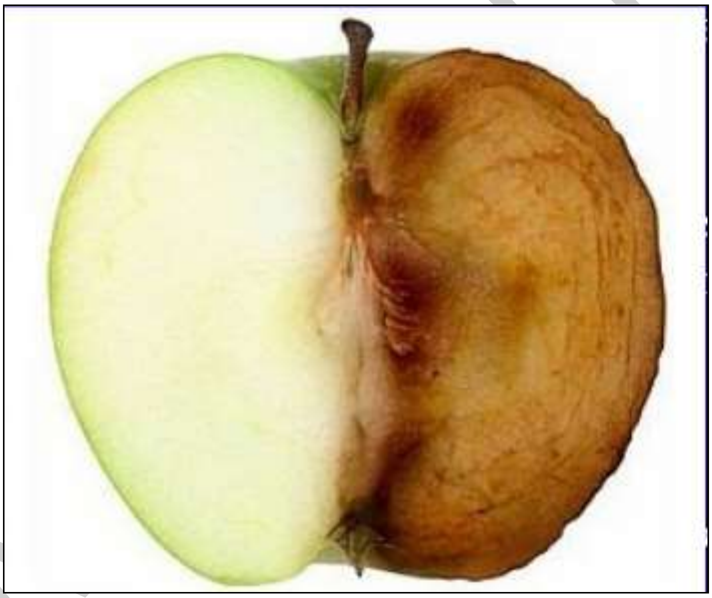
1- **Physical Food Spoilage:** is due to physical damage to food during harvesting, processing or distribution. Physical food spoilage results when moist foods are excessively dehydrated (wilting) or dried foods absorb excessive moisture.

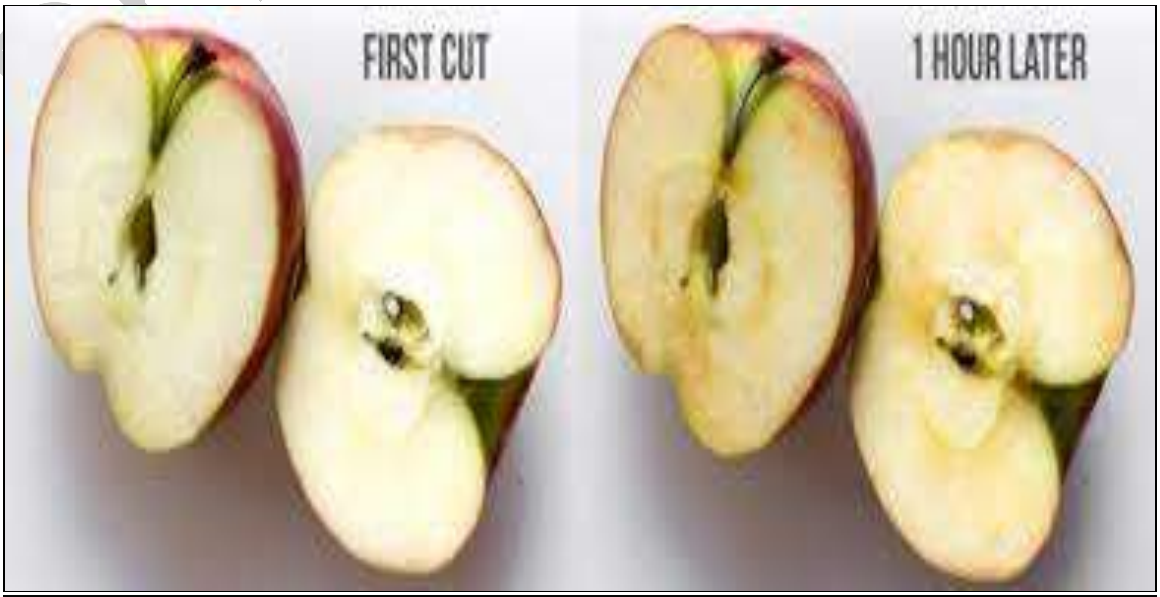
Physical Food Spoilage (Wilting)

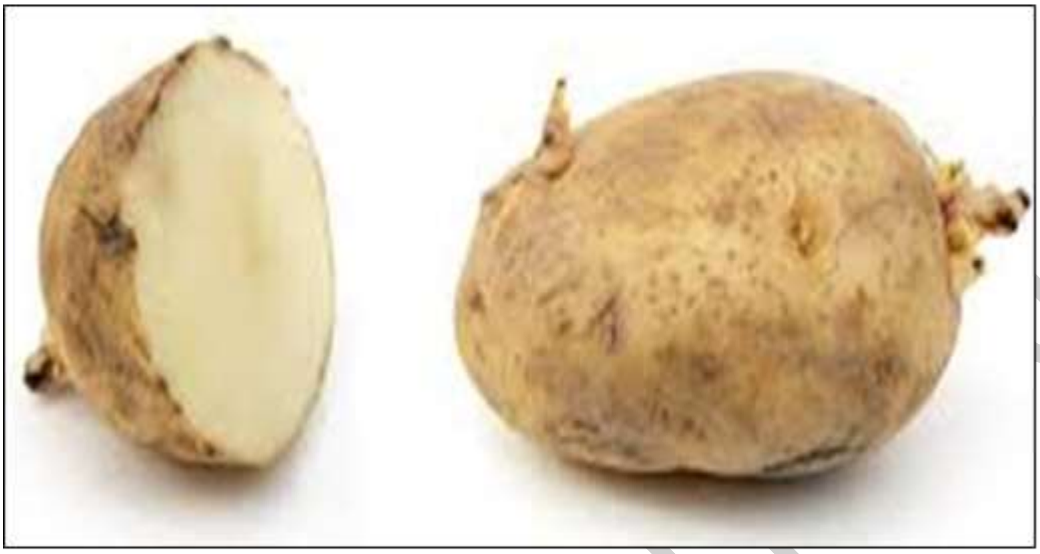
2- **Chemical Food Spoilage:** is an unwanted quality change in a foodstuff, such as self-oxidation of some compounds, enzymatic browning, discoloration, the development of off-flavours and odors (e.g. rancidity).

Examples of chemical spoilage:

(A) Oxidation of apple and potato







Why do apples and potatoes turn brown when you slice them?

- The browning reaction results from the oxidation of phenolic compounds in the fruit under the action of an enzyme called polyphenol oxidase (PPO), which is common in plant tissues.
- Once you cut the fruit, you open up some of the cells. The enzyme then has access to oxygen in the air and it does its thing, turning the fruit brown.

(B) Enzymatic Browning of Ripening Bananas

- Enzymatic browning (also called oxidation of foods) requires exposure to oxygen. Generally, it is a chemical reaction involving reaction of some enzymes (polyphenol oxidase, catechol oxidase) present naturally in food with oxygen that creates melanins (brown-black color).

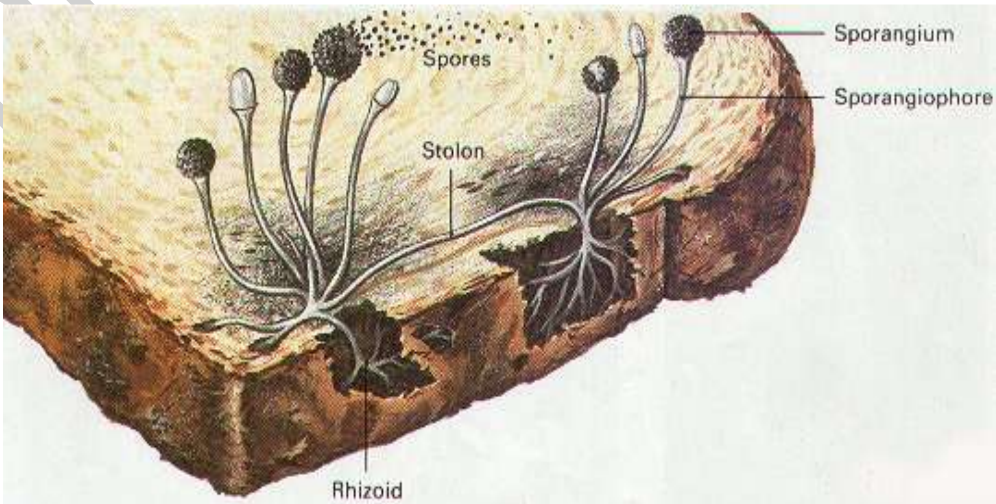
Browning of Ripening Bananas

Lab (3): Microbial Food Spoilage

Microbial Food Spoilage: occurs as a result of either microbial growth in a food or release of extracellular and intracellular microbial enzymes in the food.

- Spoilage by microbial growth occurs much faster than spoilage by microbial extracellular or intracellular enzymes in the absence of viable microbial cells.

(1) Microbial spoilage of Bread:



The Bread Mold Fungus (*Rhizopus stolonifer*)

The pictures, shows one of the most common fungi in the world, the Bread Mold Fungus, *Rhizopus stolonifer*. A few days before the picture was taken some water accidentally seeped into a package of cornmeal. When I finally opened the package, the cornmeal was spoiled and its surface was covered with the stuff appearing in the upper two-thirds of the picture.

Molds of bread:

Molds of Bread are one of the following:

- Green mold caused by *Penicillium*.
- Black mold caused by *Rhizopus stolonifer* & *Aspergillus niger*.
- Red or rose mold caused by *Monilinia*.

(2) Microbial Spoilage of Vegetables

- Vegetables are a good substrate for yeasts, molds or bacteria

- It is estimated that 20% of all harvested fruits and vegetables for humans are lost to spoilage by these microorganisms.

- Because bacteria grow more rapidly, they usually out-compete fungi for readily available substrates in vegetables.
- As a result, bacteria are of greater consequence in the spoilage of vegetables with intrinsic properties that support bacterial growth (favorable pH, Eh).

- The higher pH values of the tissues of many vegetables makes them more effected by bacterial invasion than fruits.

- The bacteria involved are usually pectinolytic species of the Gram negative genera *Erwinia* , *Pseudomonas* and *Xanthomonas* , *Clostridium*, and the non-sporing Gram-positive organism *Corynebacterium sepedonicum* .

Impact of microbial spoilage on vegetables

Microbial spoilage including the following:

- Off-flavor (e.g., fermented aroma with cut lettuce) formation.
- Slimy surface (e.g., “baby” carrots).
- Wetness and soft rot (e.g., cut bell pepper).
- Discoloration (e.g., apple wedges).
- Visual microbial growth/colonies (such as apple wedges, cantaloupe chunks, and cored pineapple).

The major fungal and bacterial post-harvest pathogens are tabulated under.

Table 1: Bacterial Vegetable Pathogens

CROP	*Pseudomonas*	*Erwinia*	*Xanthomonas*	*Bacillus*	*Clostridium*	*Lactic acid bacteria*
Broccoli	+	+	+			
Cabbage	+	+	+			
Carrot	+	+		+		
Sweet Corn						
Cucumber		+		+		
Lettuce head	+	+	+			
Lettuce leaf	+	+	+			
Onion		+		+		
Potatoes	+	+		+	+	
Tomatoes	+	+	+	+		+

Table 2: Fungal Vegetable Pathogens

CROP	*Geotrichum*	*Rhizopus*	*Fusarium*	*Alternaria*	*Colletotrichum*	*Botrytis*	*Sclerotinia*
Broccoli		+		+		+	+
Cabbage		+		+			+
Carrot	+					+	+
Cucumber		+	+		+		
Lettuce head	+					+	+
Lettuce leaf	+					+	+
Onion	+		+	+	+	+	
Potatoes		+	+				
Tomatoes	+	+	+	+	+	+	+

Sources of Contamination

1. Surface contamination – Soil, water, air, human pathogens from manure (night soil)
2. Harvesting - hand picking vs. machines
3. Packaging: containers reused
4. Markets – handling, cross-contamination

(3) Microbial Spoilage of Fruits and Fruit Products

Specific spoilage organisms:

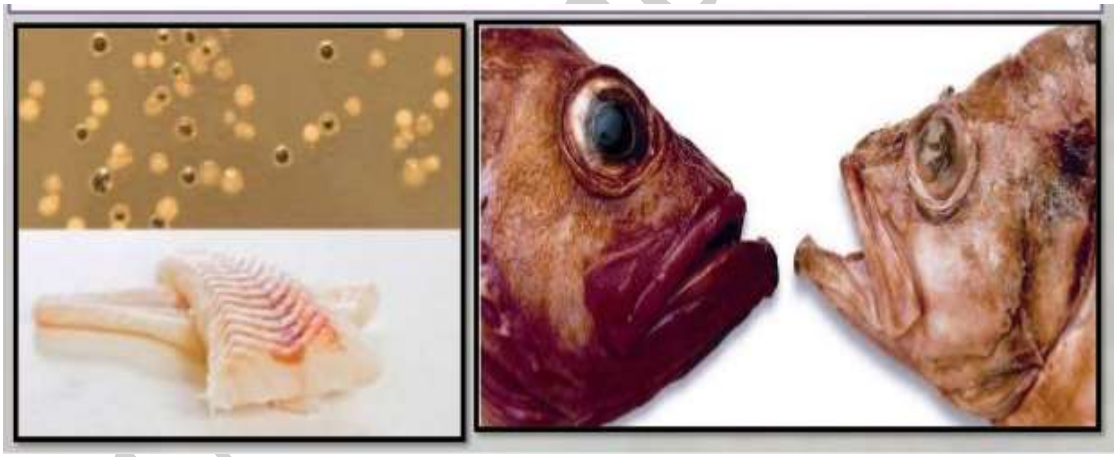
1-	Blue rot – *Penicillum* (Citrus fruits)	
2-	Downy mildews – *Phytophora*, large masses of mycelium (graps)	
3-	Black rot – *Aspergillus* (onions)	
4-	Sour rot – *Geotrichum candidum*	

Lab (4): Microbial Spoilage of other Types of Foods

(1) Microbial Spoilage of Fishes

- As a rule, fish spoil more rapidly than meat under chill conditions.

- The pH of mammalian muscle, around 5.6 is lower than that of fish (6.2 – 6.5) and this contributes to the longer storage life of meat.



Spoilage of fish can be occurred as the following:

1- Putrefaction (Breakdown of protein): occur as slime formation.

2- Rancidity (Breakdown of fats): occur as rancid odor.

3- Sourness (Production of lactic acid): occur as discoloration.

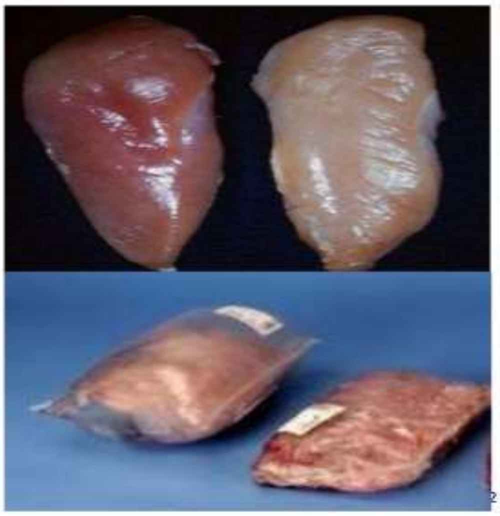
(2) Microbial Spoilage of Cheese

Cheese can be spoiled by yeasts, molds and bacteria. The most common bacterial spoilage is “slimy curd” caused by *Alcaligenes* spp. (Gram negative, aerobic rod bound in soil, water and intestinal tract of vertebrates).

Penicillum, Mucor and other fungi also grow well on cottage cheese and impart stale or yeasty flavors.

Microbial Spoilage of Cheese

(3) Microbial Spoilage of Meats



Food	Types of Spoilage	Spoilage Microorganisms
MEAT		
Fresh	Putrefaction	*Clostridium, Pseudomonas, Proteus, Alcaligenes, Chromobacterium*
	Souring	*Chromobacterium, Lactobacillus, Pseudomonas*
Cured	Mouldy	*Penicillium, Aspergillus, Rhizopus*
	Souring	*Pseudomonas, Micrococcus, Bacillus*
	Greening	*Lactobacillus* spp., *Streptococcus, Pediococcus*
	Slimy	*Leuconostoc*
Vacuum Packed	Souring Greening	*Lactobacillus, Carnobacterium, Leuconostoc*
Poultry	Odor, Slime	*Pseudomonas, Alcaligenes, Xanthomonas*

Lab (5): Food Preservation Methods

Vaccum packing

Drying

Pickling

Refrigeration

Sugaring

Salting

Canning and bottling

Food preservation is known "as the science which deals with the process of prevention of decay or spoilage of food thus allowing it to be stored in a fit condition for future use".

Preservation ensures that the quality, edibility and the nutritive value of the food remains intact. Preservation involves preventing the growth of bacteria, fungi and other microorganisms as well as retarding oxidation of fats to reduce rancidity.Knowing how to preserve food has been essential throughout our history as humans. Consider that before the advent of refrigeration, which was originally devised in the 18th century, but was not perfected and widespread until the 20th century, most of civilization had to make do without refrigeration and freezing.

Methods of food preservations

(1) Food Preservation by Drying Method

Drying, arguably the oldest food preservation method, is a great way of preserving herbs, fruits, vegetables and meats. Since the beginning of time people have let sun and nature take care of drawing moisture out of foods. This practice is used throughout the world.

For example, Southern Italy is known for drying tomatoes, while India is known for drying chilies, mangos and a host of spices.

To dry herbs, simply tie them together and hang in a sunny spot away from any humidity. To dry fruits or vegetables, set them out on a clean surface and keep them in the sun for a few weeks (this only works well in dry, warm climates). A more modern method of drying is to use an electric dehydrating machine.

Foods preserved by drying method

(2) Food Preservation by Salting Method

Salting is a sub category of the drying method. The main difference here is that salt is added to products, mainly meat and fish, to draw out moisture. This lowers the bacteria content and makes food adaptable for later use. Adding salt to animal protein turns it a bit leathery. Popular foods made in this tradition are beef jerky and dry salted cod.

Foods preserved by salting method

(3) Food Preservation by Smoking Method

Smoking is the process of flavoring, browning, cooking, or preserving food by exposing it to smoke from burning or smoldering material, most often wood.

Meats and fish are the most common smoked foods, though cheeses, vegetables, and ingredients used to make beverages are also smoked.

Foods preserved by smoking method

(4) Food Preservation by Canning Method

The canning technique was developed by a French chemist in 1795 and was used to preserve food for Napoleon's army. Canning is a popular way of preserving fruits, vegetables and meats.

In order to can foods, you need heat. Both cans and glass jars are suitable for canning. The important thing will be to sterilize your equipment in simmering water for a few minutes (this includes lids). Then they will be ready to be filled with things like jam. After filling, place the lid on firmly, but not too tight. To finalize the process lower the jars into a pot full of water, cover and bring to a boil. Process for about 10 minutes. Pull the jars out of the hot water and let cool. They will vacuum seal as they cool. Cooking times vary per recipe.

Foods preserved by canning method

(5) Food Preservation by Pickling Method

The main difference between this category and canning is that you need two things for pickling: salt and acid. Pickling requires you soak your produce, most famously cucumbers, in a brine with salt. Vegetables are the main foods which preserved by pickling method.

When they have pickled for the desired amount of time you transfer them to a jar full of vinegar. At this point you can use the canning method to produce a vacuum seal, if you wish.

A bonus of pickling is that it does not change the texture too much. The vegetables undergo a fermentation process, which also results in a vitamin boost. Pickled vegetables are known for having an increased level of vitamin B6.

Foods preserved by pickling method

(6) Food Preservation by Freezing Method

In the olden days, people would carry ice down from a neighboring mountain. Of course, now we use electric freezers to preserve our foods. Freezing changes the texture of most fruits and some vegetables, but meats and fish fair well. In the summertime, you may want to freeze your berries so that you have them available for smoothies or baking later in the year.

The best way to do this is freeze fruit in batches (the same method would apply to vegetables). For instance, scatter fresh berries on a baking tray and put it in the freezer. After they have frozen solid, put them in a bag. This will avoid clumps of berries that are impossible to separate without thawing.

Foods preserved by freezing method

Lab (6): Effect of Natural Preservatives Found in Plants on the Growth of Microbes

(A) Background:

Microorganism contamination at various stages of food chain is one of the major causes for food spoilage that leads to food waste, increasing food insecurity issues and substantial economic losses.

The detail of the losses of differ category is as follows; root crops (40–50%), fruits, and vegetables (35%), fish and seafood (30%), cereals (20%), meat, oil seed, and dairy products (20%)

Researchers and consumers are discouraging the use of synthetic preservatives due to their negative health impacts. Naturally occurring antimicrobials have gained attention among researchers and food manufacturer due to their safety and nontoxic status.

Natural preservatives are easy to obtain from plants, animals and microbes. In this regard plants are being considered as most important and rich natural source of antimicrobial substances like saponins, tannins, alkaloids, alkenyl phenols, glycoalkaloids, flavonoids, sesquiterpenes, lactones, terpenoids and phorbol esters. These plant substances act as antimicrobial, antioxidants, flavor and color enhancer. These properties of the plant agents do not only extend the shelf life of the product but also enhance the organoleptic acceptability of the products.

Table 1: Some selected plant products and their natural antimicrobial potential.

No.	Plant-based products	Target organisms
1	Clove	*E. coli*, *Pseudomonas* spp., *Clostridium* spp., *S. aureus*, *Salmonella*, *Bacillus* spp.,
2	Ginger	
3	Cinnamon	
4	Seed s of Anise	
5	Cardamom	*E. coli*
6	Grape seeds	*Listeria*
7	Peel of lemon	*E. coli*
8	Peel of pomegranate	*S. aureus*
9	Pomegranate	*Salmonella*
10	Strawberry	*E. coli*

(B) Procedures:

1- Collection of plant materials as in figures below:

Buds of Clove

Cortex of Cinnamon

Rhizome of Ginger

2- The collected plant material is crushed in small pieces 2 – 6 mm by using the cylinder crusher (figure below).

3- Fifty grams of crushed plant material were extracted with 500 ml of the solvent (distilled water, methanol, acetone, …., etc) in homogenizer, for 30 second, the product was transferred into a container to stand for about 4 hours, and then filtered through whatman no. l filter paper to obtain the clear extract (figure below).

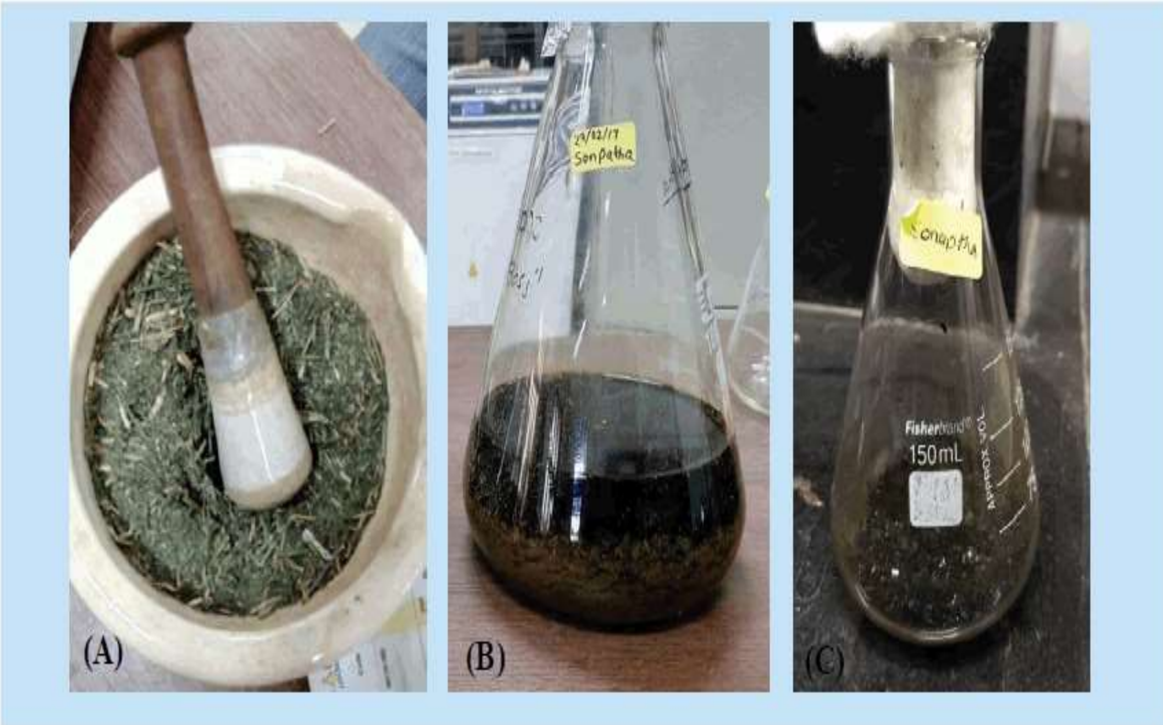


Figure 1: Preparation of crude plant extract. (A) Crushed leaves (B) Crushed leaves soaked in methanol (C) Concentrated plant extract

4- The obtained clear extracts were concentrated under reduced pressure using an evaporator to produce semi solid or solid mass under 50 °C, the data are recorded as in table below:

Table: Weights of the used plants and their extracts.

Serial No.	Plant name	Plant weight (g)	Weights of extract (g)
1.	Clove	50	4.82
2.	Cinnamon	50	5.63
3.	Ginger	50	6.12
4.	Cardamom	50	7.12

5- The microbial test organisms (bacterial and fungal test strains) were prepared for antimicrobial assay by seeding or streaking method.

6- Dispense filter paper discs (6 mm in diameter) loaded with 50µl from crude extract and drying.

7- Under aseptic conditions, using sterile forceps the loaded discs were placed onto the surface of the plates inoculated with the test organisms.

8- Put plates in refrigerator for 15 min, then the plates were incubated at 37 °C for 24 h for bacterial strains and 28 °C, 72 h for the fungal strains. After this period, it was possible to observe inhibition zone. The controls discs (discs with the solvents without plant material) were also tested.

9- Examine the plates and record the results:

- **Results**

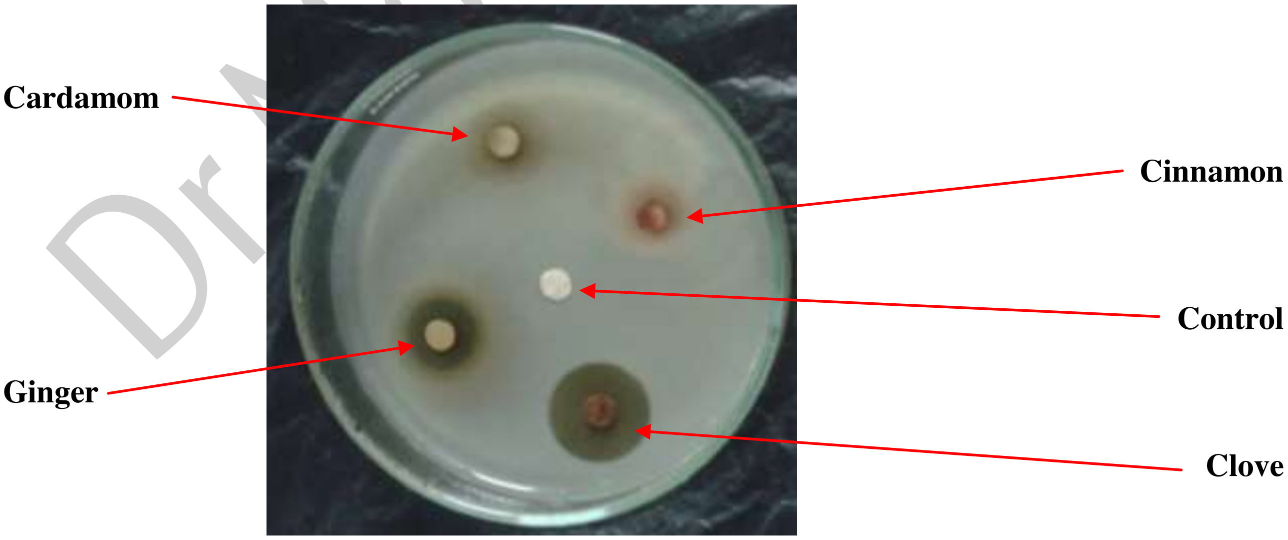


Figure: Susceptibility of *E. coli* to four plants extracted with distilled water

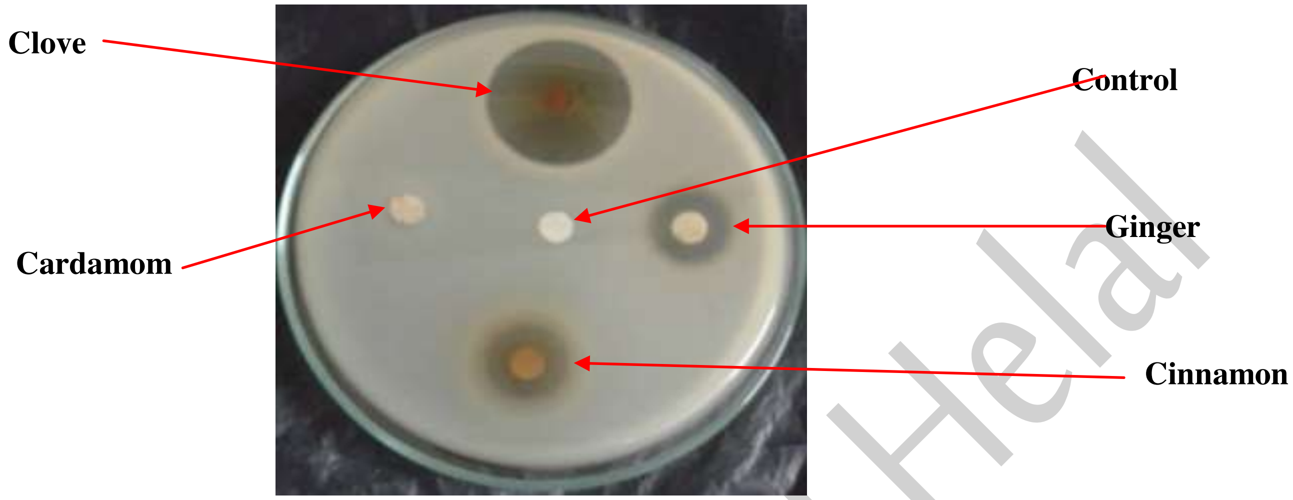


Figure: Susceptibility of *S. aureus* to four plants extracted with distilled water

The obtained results are recorded as in the following table:

Table: Susceptibility of bacterial test organisms to the different plant extracts.

No.	Test organisms	Plant extracts			
		Clove	Cardamom	Cinnamon	Ginger
1	*S. aureus*	15.0 mm	-	11.0 mm	10.0 mm
2	*E. coli*	28.0 mm	12.0 mm	8.0 mm	12.0 mm

(-): no susceptibility.

Lab (7): Fermented Food Products (Lactic Fermentation)

Definition of lactic fermentation:

Fermentation in which lactic acid is produced from carbohydrate materials (as lactose in whey) by the action of any of various organisms but especially the lactic acid bacteria.

Or:

Metabolic process by which glucose and other six-carbon sugars (also, disaccharides of six-carbon sugars, e.g. sucrose or lactose) are converted into cellular energy and the metabolite lactate, which is lactic acid in solution. It is an anaerobic fermentation reaction that occurs in some bacteria and animal cells, such as muscle cells.

Types of lactate fermentation:

(1) Homofermentative process

The homofermentative lactic acid fermentation converts a six-carbon sugar molecule to two lactic acid molecules, storing the released energy into two ATP molecules. The following equation describes this net result:

$$C_6H_{12}O_6 \rightarrow 2\ CH_3CHOHCOOH$$

(2) Heterofermentative process

Heterofermentative bacteria produce one mole of lactate from one mole of glucose as well as CO2 and acetic acid or ethanol. Examples include *Leuconostoc mesenteroides*, *Lactobacillus bifermentous*, and *Leconostoc lactis*.

$$C_6H_{12}O_6 \rightarrow CH_3CHOHCOOH + C_2H_5OH + CO_2$$

Applications

Lactic acid fermentation is used in many areas of the world to produce foods that cannot be produced through other methods. Two of the most common applications of lactic acid fermentation are in the production of yogurt and sauerkraut.

(1) Sauerkraut production:


The main type of bacteria used in the production of sauerkraut is of the genus *Leuconostoc* (genus of Gram-positive bacteria, generally ovoid cocci often forming chains and are catalase-negative). All species within this genus are heterofermentative and they are generally slime-forming.

Lactic acid fermentation of cabbage and other vegetables is a common way of preserving fresh vegetables in the western world. It is a simple way of preserving food: the raw vegetable is sliced or shredded, and approximately 2 percent salt is added. The salt extracts liquid from the vegetable, serving as a substrate for the growth of lactic acid bacteria.

Anaerobic conditions should be maintained, insofar as possible, to prevent the growth of microorganisms that might cause spoilage.

The bacteria produce lactic acid, as well as simple alcohols and other hydrocarbons. These may then combine to form esters, contributing to the unique flavor of sauerkraut.

Leuconostoc mesenteroidesinitiates grow in the shredded cabbage over a wide range of temperatures and salt concentrations. It produces carbon dioxide and lactic and acetic acids, which quickly lower the pH, thereby inhibiting development of undesirable microorganisms that might destroy crispness. The carbon dioxide produced replaces the air and facilitates the anaerobiosis required for the fermentation. The fermentation is completed in sequence by *Lactobacillus brevis* and is responsible for the high acidity.

https://www.youtube.com/watch?v=psjasIbndnA

https://www.youtube.com/watch?v=P4QivmKfQRE

(2)Yoghurt production

Yogurt also spelled yoghurt, yogourt or yoghourt, is a food produced by bacterial fermentation of milk.

Cow's milk is commonly available worldwide and, as such, is the milk most commonly used to make yogurt. Milk from water buffalo, goats, ewes, mares, camels, and yaks is also used to produce yogurt where available locally.

The sugar in milk (called lactose) is fermented to (lactic acid) and it is this that causes the characteristic curd to form.

The main method of producing yogurt is through the lactic acid fermentation of milk with harmless bacteria. The primary bacteria used are typically *Lactobacillus bulgaricus* and *Streptococcus thermophilus*.

These bacteria produce lactic acid in the milk culture, decreasing its pH and causing it to congeal. The bacteria also produce compounds that give yogurt its distinctive flavor.

For a probiotic yogurt, additional types of bacteria such as *Lactobacillus acidophilus* are also added to the culture.

Procedures:

1. Heat milk in a stainless steel (or less desirably an aluminum) pan to 80- 85 °C for 15- 20 min. with constant stirring.
2. Cool 40- 45 °C as quickly as possible and add a starter culture (or inoculum) of the mixed lactic acid bacteria.
3. Depending on the yoghurt required, the milk is either kept in pan (for stirred yoghurt) or filled into individual retail pots (for set yoghurt).
4. Incubate the milk at 40- 45 °C for 4- 6 hrs to allow the fermentation to take place.
5. For stirring yoghurt, the pan of the crude is then stirring using a sterilized spoon or ladle until the crude becomes a smooth cream that has a thick consistency without any lumps.
6. Both types of yoghurt are stored in a refrigerator at 4- 8 °C.

Steps of Yoghurt production

https://www.inoxpa.com/products/product/yoghurt-production

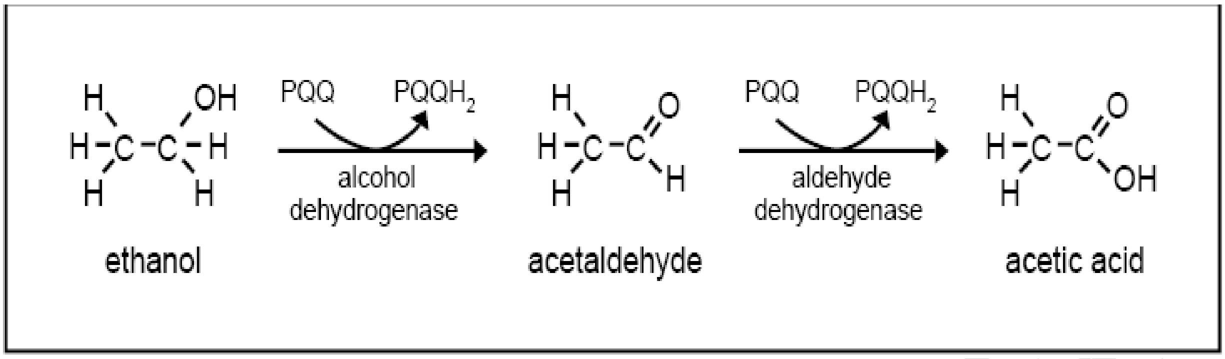
Lab (8): Vinegar (Acetic Acid) Production

Vinegar is an aqueous solution of acetic acid and trace chemicals that may include flavorings. Vinegar typically contains 5–20% acetic acid by volume.

CH_3COOH

- As old as wine production.
- French words vin (wine) and aigre (sour).
- It should contain > 4 % acetic acid in USA (FDA) and Turkey, > 5% acetic acid in EC.
- Mainly used as: flavoring agent, in food pickling, in medicine.

Vinegar is produced by oxidative (aerobic) fermentation of ethanol into acetic acid by *Acetobacter* species (ie aceti).



- Both the substrate and product are toxic: so lack of competitors during an open fermentation.

- Acetic acid bacteria: tolerate low pH and high acetic acid conc. (*E. coli* is inhibited by 1% acetic acid).

- Conversion yield of ethanol to acetic acid: 95-98 %.

- During process 2-5 % of acetic acid may be over oxidized (undesired) to CO_2 and H_2O.

- Stop oxidative fermentation before ethanol is completely depleted to prevent over oxidation.

- Leave wine (in a plate) open to atmosphere, a film (A acid bacteria) formed over the surface (sirke anası).

	Acetobacter	Film formed	Opt. temp. C	Max.acid conc.
Malt solution	*oxydans*	very thin	18-21	2
Beer	*Aceti pasteurionum*	Medium thickness	34	6
wine	*arleanense*	thick	35	9
Quick prod. bacteria	*Ascendens* *schutzenbachi*	Thin	31 25	9 11.5

Initially white colored, later turns in to dark yellow color, it is sticky and has jelatin like texture.

Usually the acetic acid is produced by the fermentation of ethanol or sugars by acetic acid bacteria. Vinegar is now mainly used in the culinary arts: as a flavorful, acidic cooking ingredient, or in pickling.

Acetic acid is made by the process of fermenting various substances: starchy solutions, sugar solutions or wine with *Acetobacter* bacteria

❖ Types of the produced vinegar

(1) Cider Vinegar

- From juice of apples which contain at least 4% acetic acid (equivalents)
- 4% = 40 grain
- 10% = 100 grain (scale for acetic acid)

(2)Grain Vinegar (Distilled)

- From distilled grain alcohol

❖ Factors affecting growth of acetic acid bacteria:

(1) **Ethanol:** should be less than 10-14 %, (if it is less than 2 %, probability of over oxidation increases)

Different cultures can tolerate different levels of ethanol;

- 6-7 % ethanol *Acetobacter oxydans, A. xylinium.*
- 9-11 % ethanol *A. aceti, A. pasteurionum.*
- 11-13 % ethanol *A. schutzenbachi.*

(some recently developed strains, can grow on 20 % ethanol to produce 20 g /100 cm^3 acetic acid).

(2) **Oxygen:** 12 l air is needed to oxidize 1 g ethanol

(3) **Temperature:** Opt. temp.: 28 – 34 C.

(4) **Other nutrients.**

❖ Production methods:

- Fast production: *A. schutzenbachi* and *A. curum*
- Slow production: *A. orleanense*

Good bacteria forms thin, silky film.

Wine, grape, raisin, fruits (apple, banana, mango, orange, ...) malt, corn syrups, honey, sweet potato, tea, rice, coffee pulp are being used as raw material.

Turkey: mainly from grape Juice or molasses.