studentguide

bread • cheese • fruit

microbewizard™
mastering microbiology!

Contents

General Information

Counting Microbe Colonies

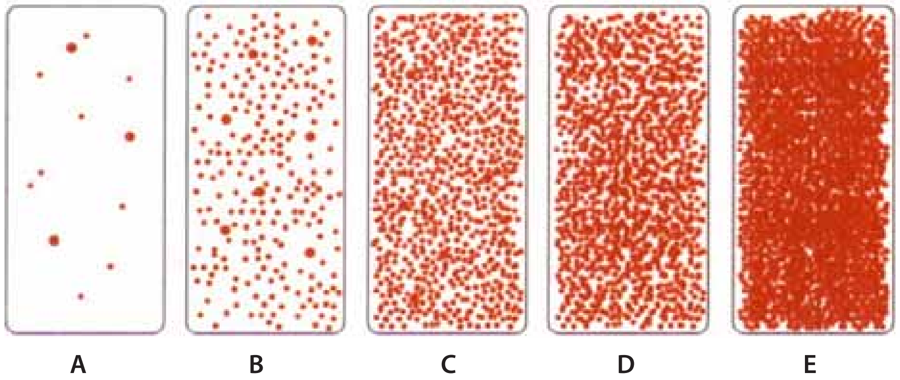


Figure 1 - Counting Panels - Making Total Colony Counts (TCC)

A 10^3 CFU/mL (1 CFU/cm^2)
B 10^4 CFU/mL (10 CFU/cm^2)
C 10^5 CFU/mL (45 CFU/cm^2)
D 10^6 CFU/mL (80 CFU/cm^2)
E 10^7 CFU/mL (100 CFU/cm^2)

NOTES: A colony Forming Unit (CFU) is a visible colony formed from usually one microbe cell or spore. TCC (total colony counts) is the total number of visible microbe colonies (growth areas) on a paddle. Paddle area is 10 cm^2. 10^4 = 10,000; cm^2 = square centimeter (area).

FOR SURFACE COUNTS: Use the corresponding Counting Panel value TCC = (CFU/cm^2)
FOR DILUTION COUNTS: Multiply the corresponding Counting Panel value by the dilution factor (10^2) TCC = panel count x dilution factor (10^2)

Sizing Microbe Colonies

Figure 2 - Sizing Microbe Colonies.
The embedded plastic stars (+) measure 4mm point-to-point. These yeast colonies (*Saccharomyces cerevesiae*) are 1-2mm in diameter. Source: Luis Camus, MD

Microslide® Anatomy

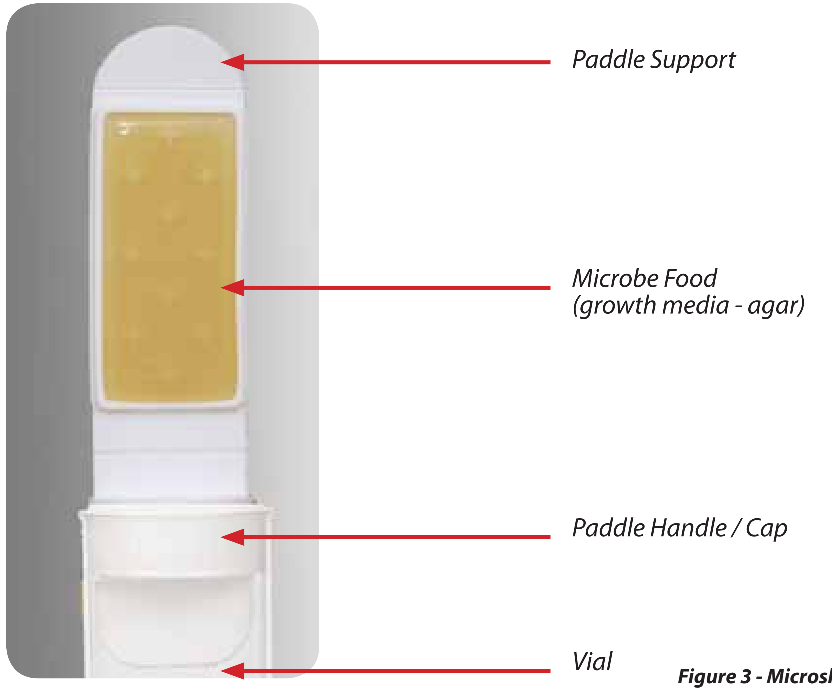


Figure 3 - Microslide® Anatomy.
Source: Luis Camus, MD

Microbes that Like

Bread

Figure 1 - Wheat, bread, wheat flour and cookies! Source: USDA

Bread loaf anatomy:
- ***Crust*** - the hard, outer, brown layer
- ***Crumb*** - the inner loaf body

Wheat bread is made of:
- wheat flour (gluten protein, starches)
- water
- yeast (fermentation cell respiration yielding alcohol and CO_2)
- added:
  - fats (to prevent "staleness"-helps retain free-water)
  - salt (helps bind proteins; less dough "stickiness")
  - preservatives (extends shelf life against microbe "spoilers")

Wheat was first planted in the US as a "hobby crop".

Today, an acre in Kansas produces enough bread to feed 9,000 individuals a day.

A bushel of wheat is equivalent to:
- 1 million individual kernels
- 60 pounds (27 kg)
- 60 pounds (27 kg) whole-wheat flour
- 42 pounds (19 kg) white flour
- 42 loaves of commercial white bread
- 90 one-pound loaves of whole-wheat bread

A commercial loaf of bread has:
- 16 oz flour (1.5 lb. / 453 g loaf)
- 24 slices

Factors Influencing Microbial Growth on Breads

Food spoilage is caused by the enzymes produced by microbes. Food spoilage microbes need: organic nutrients (proteins, carbohydrates, fats), a convenient temperature, some moisture, in most cases, some air and a suitable pH.

1. Nutrients
Breads are mostly a mixture of flour and water that is baked. Flour is a ground powder made from cereal grains (usually wheat and corn), seeds, or roots. The caloric content of flour is mostly carbohydrates (80%), proteins (15%), and fats (5%).

2. Moisture Content (water activity)
Available moisture (water) is essential for microbe growth and survival. Food microbiologists generally describe the water requirements of microorganisms in terms of the water activity (aw) of the food or surrounding environment. The water activity (aw) of a food is the ratio between the vapor pressure of the food itself, when in a completely undisturbed balance with the surrounding air media, and the vapor pressure of distilled water under identical conditions. A water activity of 0.80 means the vapor pressure is 80 percent of that of pure water. The water activity increases with temperature. Bread has a water activity of 0.96. If breads are cut or placed into a closed container (e.g. plastic bag) they will "moisture equilibrate."

Microbe Water Activity	
Most Bacteria	>0.91
Most Yeasts	>0.88
Most Fungi	>0.80

3. pH
In their natural state, most foods such as meat, fish, and vegetables are slightly acidic while most fruits are moderately acidic. A few foods such as egg white are alkaline. Breads have a pH range of 5.0 - 6.2.

Microbial cells must maintain their intracellular pH (pHi) above some critical level so that cellular proteins will not denature. Most harmful food microbes can survive within these pH ranges:

Microbes & pH	
Most Bacteria	pH 4 to 9
Most Yeasts	pH 2 to 8
Most Fungi	pH 0 to 11

4. Temperature

The most important factor directly affecting how fast microbes grow. Bread-spoiling microbes prefer a temperature range of 30˚C (86-113˚F) but can grow in temperatures as low as 5-15˚C (41-59˚F). As a rule of thumb, for every increase in temperature of 10˚C (50˚F), the activity increases two times. This rule is true within the temperature range of 32 to 60˚C.

5. Time (Shelf Life)

Bakers address the concept of time as it relates to microbial growth when a product's shelf life is determined. Shelf life is the time period from when the product is produced until the time of consumption. The "sell by" date must incorporate the shelf life of the product plus a reasonable period for consumption that consists of at least one-third of the approximate total shelf life of the perishable food product.

6. Food Additives: Preservatives

Preservatives are added to prevent the growth of unwanted microorganisms, food spoilers and food pathogens. Most bakeries add one or more of the following chemical compounds to their bread products to extend shelf life and reduce microbe contamination:

Preservatives & Microbes

Compound	Type	Antimicrobial Activity
Citric acid (acid citrates)	acidulant	BACTERIA (++) MOLDS (+) YEASTS (+)
Benzoic acid (benzoates)	preservative	BACTERIA (++) MOLDS (++) YEASTS (+++)
Sorbic acid (sorbates)	preservative	BACTERIA (++) MOLDS (++) YEASTS (+++)
Sulfites	preservative	BACTERIA (+) MOLDS (++) YEASTS (++)
Propionic acid (propionates - Ca, Na, K)	preservative	BACTERIA (++) MOLDS (++) YEASTS (+++)

Figure 2 Moldy rye bread. Can you identify this mold microbe?

Guided Investigation

Investigating Microbes on Breads Using Impression Sampling

Objectives:

- Why is bread a microbe food?
- What kinds of microbes like breads?
- What can reduce microbe populations in breads?

What You Need:

- TSA / RB Microslide® paddle [KIT]
- Bread slice [LOCAL]
- Zip-closure plastic bag [LOCAL]
- Toothpick [LOCAL]
- Magnifier [LOCAL]
- Laboratory Notebook [LOCAL]
- Digital camera (optional) [LOCAL]
- Supervising adult!

What You Do -13 Steps:

SAFETY: Remember to wash your hands after handling bread samples.

1. Take a trip to a local grocery store or bakery. Obtain a baked loaf of bread.
2. In the laboratory, use a toothpick to carefully punch 4-6 small holes in a zip-closure plastic bag.
3. Place a bread slice into a zip-closure bag and seal it. You may want to encourage bread microbes by adding moisture - a moistened piece of paper toweling or a quick spray of the bread slice with a water mister.
4. Observe the bread slice carefully over time (5-9 days) and away from sunlight. Do any visible microbes appear?
5. To direct-sample growing microbes on breads, carefully open the plastic bag so that there is enough room for the paddle to be inserted to sample a contaminated surface.
6. Twist to remove the paddle from the vial.
7. Allow the paddle surface to come into physical contact with the contaminated bread area(s). Contact both paddle surfaces on various contaminated bread areas.
8. Replace the inoculated paddle into the vial.
9. Secure the paddle with transparent sticky tape. Wash your hands!
10. Incubate the paddle for 5-7 days at room temperature away from sunlight.
11. Monitor daily for signs of colony growth - DO NOT ATTEMPT TO REMOVE THE PADDLE FROM THE VIAL. Instead, make all observations through the vial. Use a magnifier to help you observe finer details of your microbe finds!
12. Use the *Bread Micro-Community Guide* and the Counting Panels to presumptively (tentatively) identify bread microbes. Record this data in your Laboratory Notebook.
13. If you have a digital camera (iPad, iPhone or similar), use it to take close-up pictures of microbe colonies for color printing to help in later identification.

Analysis Example

A TSA/RB paddle was applied to the surface of a piece of moldy bread. The paddle was incubated at room temperature (70˚F / 20˚C) for 4 days. Use the *Bread Micro-Community Guide* to identify the bread microbe growing on both the TSA and RB paddle agars:

Figure 3 - Hunting black bread mold.

A TSA/RB paddle was applied to the surface of a piece of moldy bread. The paddle was incubated at room temperature (70˚F / 20˚C) for 5 days. Use the *Bread Micro-Community Guide* to identify the bread microbe growing on both the TSA and RB media. Source: Luis Camus, MD

DATA TABLE 1
Investigating Microbes On a Bread Slice

Paddle Agar	*Tentative Microbe Identification*	*Environmental Conditions/Notes*
RB		
TSA		

Designing Experiments

Remember that an experiment is a process or study that results in data (information). The results of experiments are not known in advance. Experimental design is a process of planning a study (experiment) to meet specified objectives. When designing an experiment, follow the scientific method - a process involving:

- Formulating a question
- Making an educated guess (a hypothesis)
- Testing your guess (experimenting)
- Analyzing your results
- Drawing conclusions (gaining knowledge; being able to explain)

After conducting investigations, you should be able to answer these investigational objective questions:

- Why is bread a microbe food?
- What kinds of microbes like breads?
- What can reduce microbe populations in breads?

More Ideas! (open inquiry)

Try these additional activities using either bread slices or additional Microslide® paddles to capture and sample bread microbes.

1. Microbe Race! Create these experimental bread slice setups: commercial bread slice (with preservatives) against a "home-baked" bread slice (without any added preservatives). Which bread will "win" (see evidence of microbe spoilage) the microbe race?

2. Mold Race 2: Which experimental condition makes it more likely for microbe spoilage to occur - An un-opened loaf of bread, or one purchased at the same time with 10 small toothpick holes poked into it?

3. Do stale (dried out) bread slices provide longer protection against microbe spoiling?

4. Which spoils more rapidly - a slice of white bread or a slice of toasted white bread after being placed in a zip-seal bag?

Questions continued....

5. Which configuration (inside a sealed zip-seal bag) is more sensitive to microbial spoiling - a baked un-cut loaf or a baked sliced loaf?

7. Compare different bread types for rate of microbe spoilage: white, brown, whole wheat-meal, bagel)

8. Compare the effects of temperature on bread microbe spoiling. Use the same type of bread, incubated under identical conditions, at different temperatures (refrigeration, room, warm).

9. How long (past its "Best Used By" date) will a commercial bread loaf (with preservatives) remain "fresh" - with evidence of microbial spoilage?

Going Further

Hunting for Bread Microbes - making sourdough bread starter
Attract yeast microbes by making your own sourdough bread starter - confirm your finds using a Microslide® paddle!

Figure 4 - Sourdough Bread Starter - Day 3
Notice the bubbles of carbon dioxide forming - a sure sign of microbe activity! Source: Kenneth G. Rainis

What You Need:

- Kitchen measuring cup [LOCAL]
- Whole wheat or pumpernickel flour (whole grain flours contain more microbe-friendly ingredients and moisture!) [LOCAL]
- Glass or plastic container (4 cup measure, 240cc) [LOCAL]
- Cool, non-chlorinated water (bottled water) [LOCAL]
- Wooden mixing spoon [LOCAL]
- Handkerchief [LOCAL]
- Popsicle stick (or similar) [LOCAL]
- Sticky tape (roll) [LOCAL]
- Warm incubation environment (68 - 70˚F / 20 - 21˚C) [LOCAL]
- Supervising Adult!
- Unopened (STERILE) TSA /RB Microslide® paddle [KIT]
- Digital camera (optional) [LOCAL]

What You Do - 12 easy steps:

1. (DAY 1) Combine 4 ounces (1 cup / 240g) whole wheat (or rye) flour with 4 ounces (1/2 cup / 120g) cool, non-chlorinated water in a container.

2. Use a mixing spoon to stir everything together. Make sure there is no dry flour anywhere. Cover the mixture with a handkerchief and allow to sit at room temperature (68 - 70˚F / 20 - 21˚C) for 24 hours.

3. (DAY 2+) Uncover, and observe the surface. Do you see evidence of bubbling? Bubbling is a sign of natural yeasts that have found a new food source and are beginning to multiply! Do you smell a fruity aroma? Again, this is evidence of growing natural yeasts.

   NOTE: Incubation of your starter at lower temperatures will delay microbial activity.

4. (DAY 3-4) To direct-sample your growing natural yeast micrommunity use a popsickle stick to scoop up a small amount (dime-size) of sourdough starter.

5. Open a Microslide® vial and add 40mL bottled (non-chlorinated) water to the fill-line.

6. Insert the popsicle stick (with sourdough starter) into the water in the vial and mix thoroughly to dissolve the flour and suspend the growing yeast cells.

7. Dip the paddle into the water sample and allow for at least a 15 second contact time.

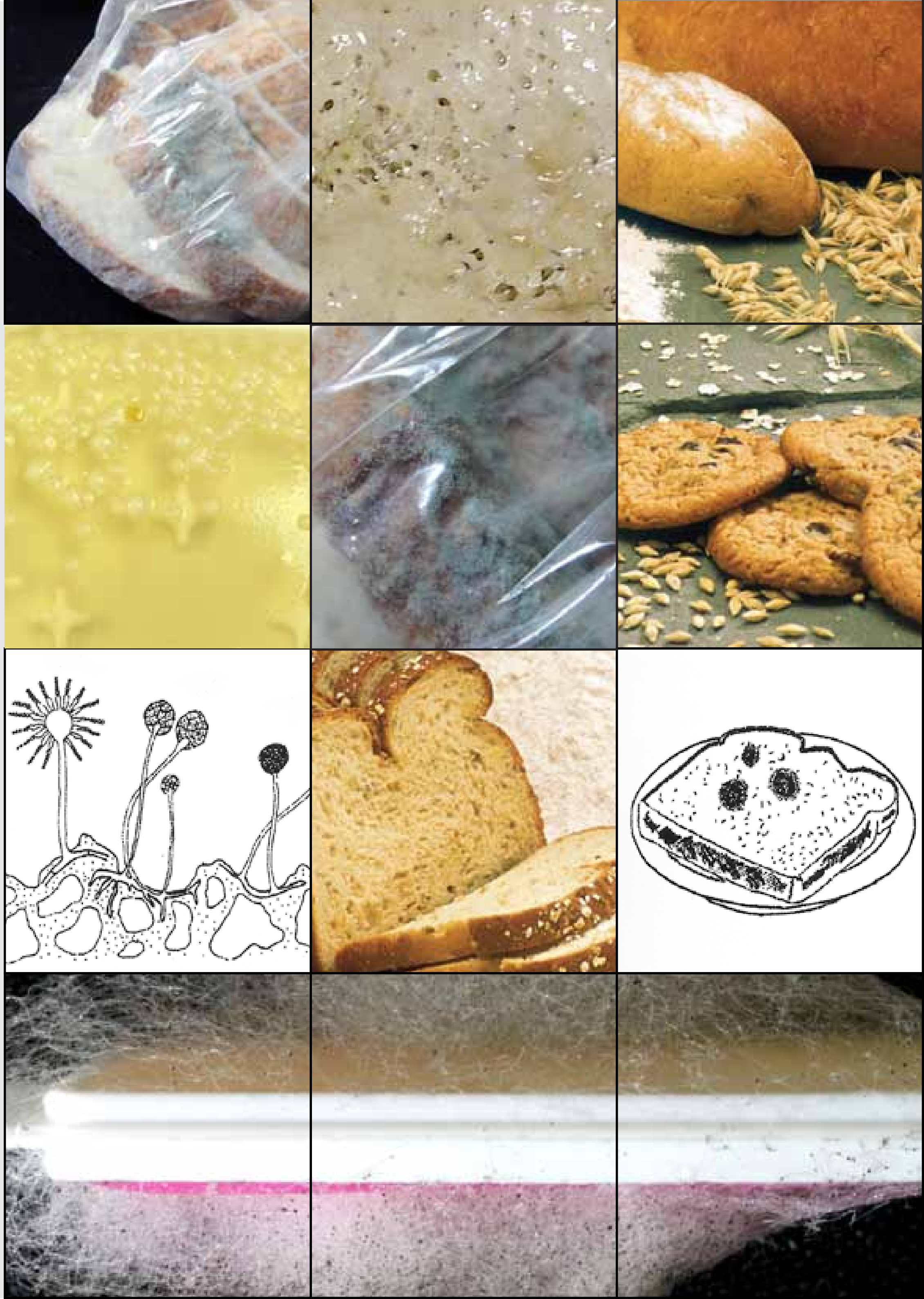
8. Remove the paddle. Empty the water in the vial, and replace the paddle.

9. Incubate at room temperature (68- 70˚F / 20 - 21˚C) for 3-5 days.

10. Monitor daily for signs of colony growth - DO NOT ATTEMPT TO REMOVE THE PADDLE FROM THE VIAL. Instead, make all observations through the vial. Use a magnifier to help you observe finer details of your microbe finds!

11. Use the *Bread Micro-Community Guide* and the Counting Panels to presumptively (tentatively) identify bread microbes. More than likely you have captured *Saccharomyces spp.* yeasts. See Figure 4. Record this data in your Laboratory Notebook.

12. Use a digital camera (iPad, iPhone or similar device) to take close-up images of microbe colonies to view or color print them for later identification.



Microbes that Like

Cheese

Cheese Story

Cheese making can be described as the process of removing water, lactose and some minerals from milk to produce a concentrate of milk fat and protein. The essential ingredients of cheese are milk, coagulating enzyme (rennet), microbe (bacterial) cultures and salt. Rennet causes the milk proteins to aggregate and ultimately transform fluid milk to a semi-firm gel. When this gel is cut into small pieces (curds), the whey (mostly water and lactose) begins to separate from the curds. Acid production by bacterial cultures is essential to aid expulsion of whey from the curd and largely determines the final cheese moisture, flavor and texture.

Blue Cheese

The origin of mold-ripened cheese is lost in antiquity. It was made in France at least as early as the Roman era. The name 'Roquefort' first appeared in the year 1070. Roquefort cheese is made from ewes' milk, and the trade name is protected throughout the world. Other cheese varieties that are ripened by the mold microbe *Penicillium roqueforti* include Blue (Bleu, Blue-veined), Gorgonzola (Italy), Stilton, Wensleydale and Dorset Blue (Blue Vinney) of England, Niva of Czechoslovakia, Danablu and Mycella of Denmark, Nuworld, U.S. and Errmite, Canada.

Camembert Cheese

A soft, creamy, surface-ripened cow's milk cheese. It was first made in 1791 at a farm in Camembert, Normandy (in northern France). In the late 19th century, wooden boxes which were used to carry the cheese and helped to send it for longer distances, in particular to America, where it became very popular. These boxes are still used today.

Cheddar

Semi-hard, pale yellow to off-white (unless artificially colored); sometimes sharp-tasting. Originating in the English village of Cheddar, England as far back as the 12th century.

Colby

Colby cheese was named after a township in Southern Wisconsin in the 1880s. Colby is high moisture, open-textured, soft-bodied and quick-curing. It is sometimes called Farmer's cheese.

Cream cheese

Cream cheese is made by acid coagulation from cream or milk to which cream has been added. Various versions (recipes) originated in England and France in the mid 1700's.

Feta Cheese

A soft-curd ancient cheese made from sheep and goat's milk. The earliest references to cheese production in Greece date back to the 8th century BC along with the technology used to make cheese from sheep's/goat's milk, as described in Homer's Odyssey.

Fresh Cheese

There are four principal types of acid coagulated fresh cheese: Cottage cheese (North American), Quark types such as Baker's cheese (European), Cream cheese, and heat-acid precipitated types including Paneer (India) and traditional Queso Blanco (Latin American). These cheese types are all made by acid coagulation of caseins rather than rennet coagulation. Cottage cheese, quark and cream cheese are normally acidified by lactic fermentation while Paneer and traditional Queso Blanco are acidified by the addition of organic acids to hot milk.

Gouda

Gouda cheese is named after the Dutch city of Gouda, not because it is produced in or near the city, but because it has historically been traded there. The first mention of Gouda cheese dates from 1184, making it one of the oldest recorded cheeses in the world still made. Gouda cheese is best described as a style of cheese making rather than a kind of cheese, as its taste depends almost completely on the time it has aged.

Paneer

Paneer has been made in India for generations, mainly in the home. Milk is coagulated by lime juice, citric acid solution, sour whey, or LAB cultures.

Process Cheese

Originated in Germany in 1885; independent development in U.S. resulted in American patent in 1917 by J.L. Kraft. Process cheese is a medium acid food with relatively high moisture content, which means that strictly speaking, it should be sterilized before storing and distributing at ambient temperature.

Provolone

The manufacturing procedures for Pasta Filata types (mozzarella and provolone) are similar. These cheeses are made using the principle of working or kneading the curd to produce the desired melting and stretching properties.

Ricotta Cheese

Ricotta cheese is made from heat-acid precipitation of proteins from whey or whey-milk blends. Like other whey cheeses, it is made by coagulating the keratin proteins that remain after casein has been used to make cheese. Thus, ricotta can be consumed by individuals who are casein intolerant.

Romano

American and Canadian term for a class of Italian hard, salty cheeses (suitable fro grating) made from cow, goat, or sheep milk, aged at least 5 months.

Swiss Cheese

Swiss (Emmentaler) cheese was first made in the fifteenth century in the Emmental Valley. Swiss type cheese made in other areas are known by local names: Gruyere (Switzerland), Allfauer Rundkase (Bavaria), Battlematt (Switzerland), Fontina (Italy), Traanon (Switzerland) and Samso (Denmark). Swiss is traditionally made in large 50 kg wheels.

The distinctive feature of Swiss cheese is the formation of eyes by the gas forming bacteria, *Propioni bacterium shermanii*. The manufacturing procedure is designed to provide the right chemical composition for the growth of *P. shermanii* and the right texture (sufficient elasticity) for bubble formation.

Terms

Aged Cheese that has been aged over 10 months to 1 year.

Bloomy rind White, velvety ring usually found on soft cheeses. It is produced by spraying the cheese surface with *Penicillium candidum* spores.

Blue (veined) Cheese treated with *Penicillium spp*. and perforated with needles which allow air to enter the cheese body and promote the growth of molds.

Brine Solution of salt water in which some cheeses are stored.

Curd The solids formed during coagulation.

Enzyme A substance used to promote the curdling of milk (e.g. rennet).

"Eyes" Holes and other openings in the cheese

Extra aged Cheese that has been aged over 2 to 7 years but not designated by year.

Ferment Biological process of lactic acid bacteria that converts milk sugar (lactose) into lactic acid and that promotes curd formation as well as contributing to the development of flavors.

Flavored Cheese that has particulates or flavoring added. Examples: Monterey Jack, Feta, Cheddar and Cream Cheese.

Mixed rind Blend of microbes (molds / bacteria) used to provide rind structure for flavor and aroma.

Pasteurization The process by which milk is heated to high temperatures to destroy certain bacteria. Most cheeses are made with pasteurized milk.

Rind Protective external layer of a cheese. Rinds can be naturally or artificially created, and either washed or brushed.

Ripened Describes a cheese whose body has softened throughout and whose flavor has developed distinctive qualities.

Salting A step, prior to or following pressing, where cheese is dry-salted or immersed in brine.

Vacuum packing A method of packaging where cheese is placed in a plastic envelope, the air is removed and the package is sealed. This prevents the cheese from drying out and restricts mold growth.

Washed rind Process by which the rinds of certain cheeses are washed periodically during ripening, resulting in the coppery or beige color. Examples: Mamirolle and Oka.

Whey Liquid that separates from solids when milk coagulates. Whey contains lactose and mineral salts.

Microbe Wizard™ Cheese Facts

Figure 1 - Cheddar cheese curds (solids) and whey (liquid) Source: USDA

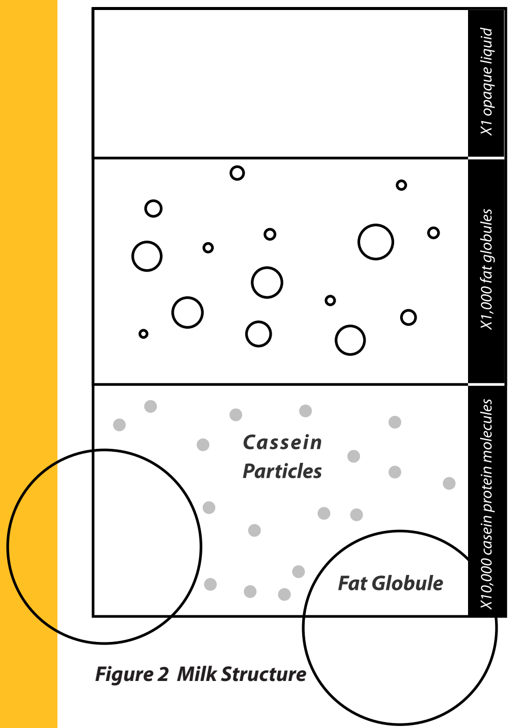


Figure 2 Milk Structure

- Cheese is an ancient food derived from animal milk by coagulation (turning into a solid curd) of the milk protein casein.
- Casein is solidified by adding the enzyme rennet and/or by the action of acid-producing microbes.
- Casein is a unique molecule - it contains at least 10 times as much water as any other protein!
- Microbes help produce a wide range of flavors and textures to cheeses during aging.
- In the U.S., more than 600 varieties of cheese are produced, largely from cow's milk.
- Milk is an opaque liquid - with large fat particles (molecules) floating about with fine casein protein particles in a watery environment, being a mixture of fine particles that do not settle out (what scientists call an emulsion; see Figure 2). That's why milk is always white - nothing ever settles out!
- The special bioenzyme rennet (chymosin) is made in the 4th stomach (abomasum) of the milk-fed calf. The practice of cheese making probably began when somebody discovered that milk stored in bags made from calf stomachs formed a sweet curd!
- Acid coagulation is used in the production of cottage cheese and bakers cheese as well as other fermented milk products such as yoghurt, commercial buttermilk, kefir etc.

Cheese categories include:

BLUE / GREEN	- flavor-producing microbes (Roquefort cheese)
HARD	- well aged (e.g. Parmesan, Romano, Swiss)
PASTA FILATA	- heated curds are stretched and molded (e.g. string Mozzarella cheese)
PROCESSED	- heated blends of fresh and aged cheeses (e.g. individual packaged slices)
SEMI-HARD	- firm (e.g. Cheddar, Colby (Farmer's) cheese)
SEMI-SOFT	- made with whole milk, microbe-ripened (e.g. Muenster cheese)
SOFT / FRESH	- made with acid (LAB) and other microbes (e.g. Cottage and Cream cheese)
SOFT / RIPENED	- made with acid (LAB) and other microbes (e.g. Camembert, Brie, Feta cheese)

Factors Influencing Microbial Growth IN and ON Cheese

Natural Fermentation of Milk

Milk is a perfect growth medium for microbes! Fermentation is a biological process that involves the production of acid by microbes (lactic acid bacteria - LAB). Over a period of 1-5 days these microbes multiply, producing acid (lowering milk pH). These microbes set the stage for others which thrive in acidic conditions, producing compounds (enzymes) that break down milk proteins creating unique flavors (and gases like carbon dioxide) - a process called ripening.

Microbes and Cheese

Lactic acid bacteria (LAB) and other microbes are present as 'contaminants' in cheese milk and further environmental contamination takes place during cheese manufacture. Provided the milk is not chilled, it is possible to make cheese without any additional cultures, but normal practice is to add domestic cultures for the manufacture of cheese from both raw and pasteurized milk.

Information Table 1
Lactic Acid Microbes Used in Cheese Making

Culture Type	Microbe	Cheese
MESOPHILIC CULTURES (moderate temperature microbes)	*Lactococcus lactis types*	common close (small) curd; acidity without gas; flavors Cheddar, Colby, Feta, Monterey Jack, Camembert, Cottage
	Lactobacillus spp. types *Leuconostoc lactis*	Produce CO_2; used for making cheese Leuconostoc lactis with small holes
THERMOPHILIC CULTURES (warm temperature microbes)	*Streptococcus salivarius*	used in Swiss and Italian cheese
	Lactobacillus bulgaricus	used in Provolone and yoghurt
	Lactobacillus helveticus	used in Swiss and Italian cheese
	Lactobacillus delbrueckii	used in yoghurt
	Bifidobacterium spp.	used in yoghurt
	Streptococcus thermophilus	used in yoghurt; ripening (Swiss, Italian cheese)

Inocula of bacteria, yeasts and molds added to cheese milk and cheese. In the broadest terms, cultures have two purposes in cheese making: 1) to develop acidity; and 2) to promote ripening. Lactic acid cultures contribute to both of these functions. Cultures can be broken into two types: Mesophilic and Thermophilic. Mesophilic (non-heat loving) culture used for making 90% of cheese varieties that are not heated to more than 102 degrees. They include: Soft, Blue, Feta, Cottage, Farmers, Colby, Cheddar, Camembert, Brie, cultured Buttermik and sour cream. Thermophilic (heat-loving) culture used to make cheese that can be heated to 130 degrees. They include: Parmesan, Provolone, Mozzarella, Swiss, Monterey Jack cheese and yogurts.

Information Table 2
Other Microbes Used in Cheese Making

Cheese	Microbe (kind)	Where to Look
BLUE / GREEN Bleu de Bresse (Bress Blue)	*Penicillium roqueforti* [MOLD] *Penicillium camemberti* [MOLD]	white outer rind (*P. camemberti*) interior (*P. roqueforti*)
HARD Swiss, Parmesian, Romano	*Propionobacter shermanii* (*Brevibacterium linens*)	gas producer eye (hole) bigger the eye, the more flavor outer rind
SOFT-RIPENED Brie (Camembert) & Langres	*Penicillium candidum* [MOLD]	white outer rind
SOFT-FRESH Yoghurt; Kefir	[LAB BACTERIA]	curds and whey
SEMI-SOFT Muenster	*Brevibacterium linens* [BACTERIA]	orange outer rind

Microbes and Acidity (lower pH)
Acid development in cheese making is absolutely essential to cheese flavor, cheese texture and cheese safety. Acid is required to:

- Assist in curd formation (coagulation).
- Prevent growth of spoilage microbes
- Prevent growth of pathogenic (disease-causing) microbes
- Develop cheese texture, flavor and color

High pH produces soft, soapy, fruity and bitter cheese. Low pH produces cheese with a brittle texture and mottled color

Microbes and Curing
Growth factors produced by microbes contribute to:
- Interior ripening
- Flavors
- Texture

Spoilage Microbes
Spoilage is the deterioration of food to a point where it is inedible to humans and/or its quality or freshness makes it unusable. Food that is capable of spoiling is termed perishable.

Information Table 3
Some Cheese Spoilage Microbes

Cheese	Microbe (kind)	Where to Look
Cottage Cheese	*Bacillus spp.* [BACTERIA] *Micrococcus spp.* [BACTERIA] *Pseudomonas spp.* [BACTERIA] *Geotrichum candidum* [YEAST] *Penicillium spp.* [MOLD]	Solids and liquids
Cream & Processed Cheese	*Bacillus spp.* [BACTERIA] *Rhizopus spp.* [MOLD] *Aspergillus spp.* [MOLD]	Surface
Soft. Fresh Cheese	*Lactococcus spp.* [BACTERIA] *Micrococcus spp.* [BACTERIA] *Pseudomonas spp.* [BACTERIA] *Rhizopus spp.* [MOLD] *Aspergillus spp.* [MOLD]	Solids and liquids
Ripened Cheese	*Lactococcus spp.* [BACTERIA] *Rhizopus spp.* [MOLD] *Aspergillus spp.* [MOLD]	Surface

Source: Kenneth G. Rainis

Guided Investigation

Investigating Microbes In & On Cheese

Objectives:

- Determine cheese category types
- Identify the kinds of microbes that grow IN and ON cheese

What You Need:

- TSA / RB Microslide® paddle [KIT]
- Cheese sample [LOCAL]
- Magnifier [LOCAL]
- Laboratory Notebook [LOCAL]
- Digital camera (optional) [LOCAL]
- Supervising adult!

Figure 3 - Cheddar cheese curds (solids).

Source: Kenneth G. Rainis

What You Do -12 Steps:

SAFETY: Remember to wash your hands after handling cheese samples.

1. As your teacher directs, take a trip to a local grocery store or delicatessen. Obtain a cheese sample(s).
2. In the laboratory, classify your cheese sample (determine its category).
3. Observe the cheese. Use a digital camera to photograph it.
4. Record observations in your Laboratory Notebook.
5. Does the interior of your cheese sample have "eyes" (holes)? Record observations in your Laboratory Notebook.
6. Use the Information Tables (1 and 2) as a guide as to what microbe(s) to expect.
7. Follow this standard sampling procedure:
   a. Twist to remove the paddle from the vial.
   b. Allow the paddle surface to come into physical contact with the cheese surface. Contact both paddle surfaces on various cheese surfaces (e.g. interior and exterior).
   c. Replace the inoculated paddle into the vial.
8. Secure the paddle with transparent sticky tape. Wash your hands!
9. Incubate the paddle for 5-7 days at room temperature away from sunlight.
10. Monitor daily for signs of colony growth - DO NOT ATTEMPT TO REMOVE THE PADDLE FROM THE VIAL. Instead, make all observations through the vial. Use a magnifier to help you observe finer details of your microbe finds!
11. Use the *Cheese Micro-Community Guide* and the Counting Panels to presumptively (tentatively) identify cheese microbes. Record this data in your Laboratory Notebook.
12. Use a digital camera (iPad, iPhone or similar device) to take close-up images of microbe colonies for viewing and/or color printing for later identification.

Analysis Example

A TSA/RB paddle was applied to the outer and interior surface of Camembert cheese. The paddle was incubated at room temperature (70˚F / 20˚C) for 4 days. Use the *Cheese Micro-Community Guide* to identify cheese microbes:

Figure 4 - Slice of Camembert cheese. For soft, fresh cheeses (like cottage or mozzarella), immerse the paddle directly into the package container to sample the cheese surfaces and the watery whey. Source: Kenneth G. Rainis

DATA TABLE 1
Investigating Microbes On a Bread Slice

Paddle Agar	***Tentative Microbe Identification***	***Cheese Category / Notes***

Designing Experiments

Remember that an experiment is a process or study that results in data (information). The results of experiments are not known in advance. Experimental design is a process of planning a study (experiment) to meet specified objectives. They should follow the scientific method – a process involving:

- Formulating a question
- Making an educated guess (a hypothesis)
- Testing your guess (experimenting)
- Analyzing your results
- Drawing conclusions (gaining knowledge; being able to explain)

After conducting investigations, you should be able to answer these investigational objective questions:

• Determine cheese category types
• Identify the kinds of microbes that grow in & on cheese.

Answer these Questions:

1. Why is cheese a microbe food?

2. What kinds and types of microbes are used in the cheese-making process?

3. What kinds of microbes are cheese spoilers?

More Ideas! (open inquiry)

As your teacher directs, try these additional activities using either process cheese pieces (or slices) or additional Microslide® paddles to capture and sample cheese microbes.

Microbe Race! Create these experimental processed slice setups in moisture bags: commercial cheese slice (in a zip-closure bag bath with a small amount of misted water). How long does it take various types of cheeses to spoil in a high-moisture environment?

What makes limburger cheese stink?

Going Further

Making Cottage Cheese

Use buttermilk as a culture starter to make cottage cheese!

Figure 5 Cottage Cheese
Using buttermilk as a starter produces small-curd cottage cheese!
Source: Kenneth G. Rainis

What You Need:

- 1 Gallon (3800 mL), fresh whole milk (cow or goat) - not ultrapasteurized [LOCAL]
- 1/2 Cup (240mL), buttermilk [LOCAL]
- 1 Teaspoon (5 g) non-iodized salt (kosher or sea salt) [LOCAL]
- 2 Large squares (12-inches / 30cm), cheesecloth [LOCAL]
- 1 Large pan: 6-8 quart size (6 - 7 L)(non-aluminum) [LOCAL]
- Kitchen thermometer [LOCAL]
- Canning pan (large enough to accommodate pan, above) [LOCAL]
- Stainless steel spoon, long handle [LOCAL]
- Colander [LOCAL]
- Long-bladed knife (cut curd) [LOCAL]
- Supervising Adult!
- Access to a kitchen stove or hot plate [LOCAL]
- Unopened (STERILE) TSA /RB Microslide® paddle [KIT]
- Digital camera (optional) [LOCAL]

What You Do:

STEP:

1. (DAY 0) Pour milk into a 6-8 quart (6 - 7 L) pan.

2. On a stove (or hot plate) heat the milk to 75˚F (24˚C) and then remove from heat. Use a kitchen thermometer to monitor the temperature.

3. Pour in the buttermilk starter culture. Stir. Cover with cheesecloth. Allow to sit undisturbed for 24 hours at 75˚F (24˚C).

4. (DAY 1) Remove the cheesecloth. The milk will have set to a custard-like consistency (curd) and may have a covering of liquid on the top (whey).

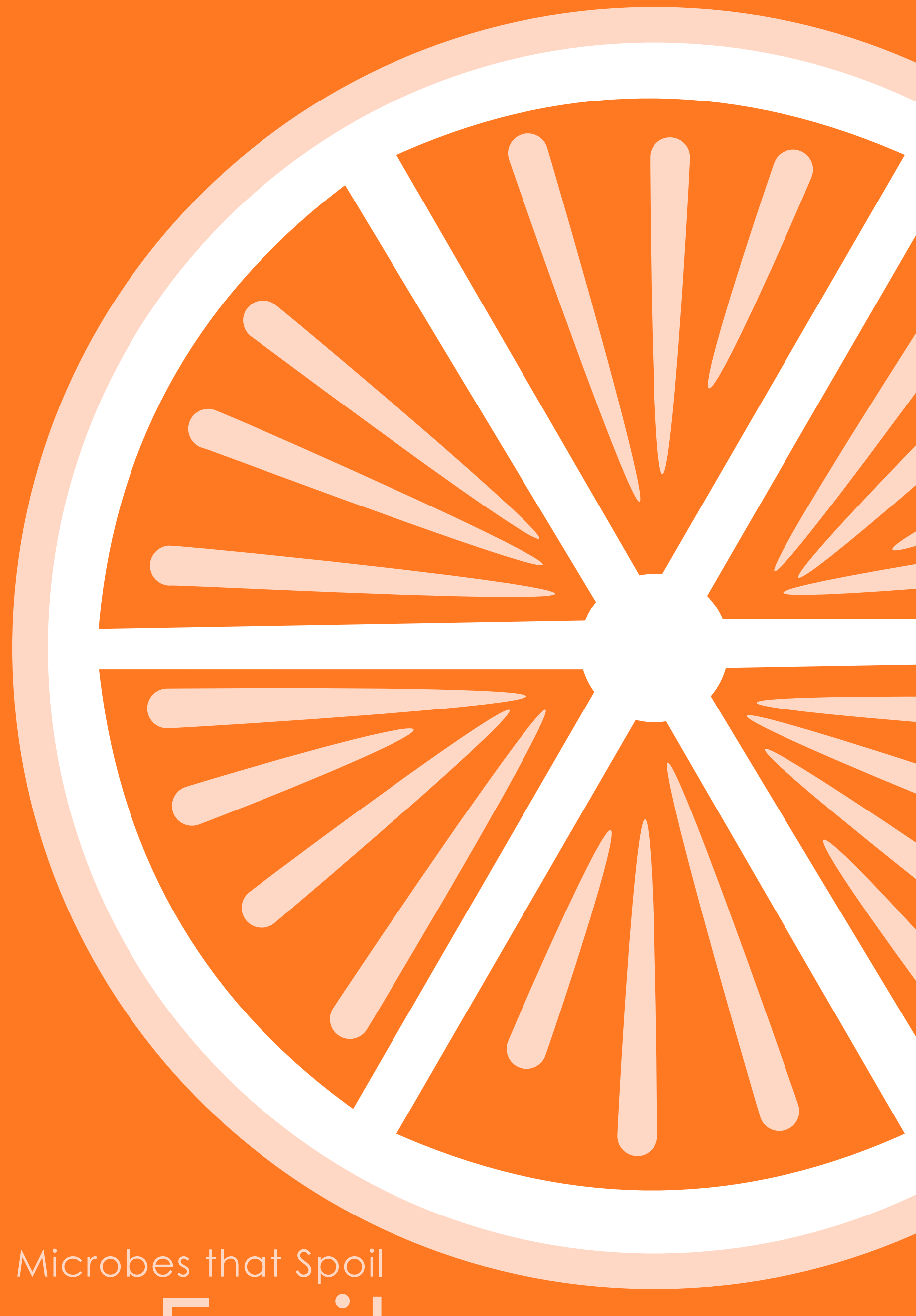
5. Using a knife, cut the curd into 1/2-inch cubes.

6. Place the thermometer in the pan of curds. Place the pan of curds in the larger pan so that the water level surrounding the pan reaches the level of the curds inside. Gently stir the curds for 30 seconds every 5 minutes or so as the temperature of the curds slowly rises. Gentle stirring keeps the curds from sticking together (matting). When the curds reach 100°F (37.8˚C), increase the heat until the curds reach 120°F (48.8°C). Hold the curds at this temperature for 25 to 30 minutes, stirring more vigorously every 5 minutes. Most of the curds will be firm now. You can squeeze a few curds to see if they are still soft in the center. (A little soft is OK; runny is not.) If curds are not firm enough, continue to hold at 120°F (48.8°C) for an additional 5 minutes.

7. Place a double thickness of cheesecloth in a colander to line it.

8. Carefully pour the curds into the colander to drain off the whey. Let drain for 5 minutes.

9. Place the curds in a bowl. Stir in salt. Refrigerate. Enjoy within a week!



Microbes that Spoil

Fruit

Microbe Wizard™ Fruit Facts

Figure 1 - Spoiled Apples. Source: Kenneth G. Rainis

- ***Consumption of fruit and vegetable products has dramatically increased in the United States by more than 30 percent during the past few decades.***
- ***The USDA (US Dept. of Agriculture) estimates that about 20 percent of all fruits and vegetables produced are lost each year due to microbe spoilage.***
- Spoilage is the deterioration of food to a point where it is inedible to humans and/or its quality or freshness makes it unusable. ***Food that is capable of spoiling is termed perishable.***
- ***Most microbes initially observed on whole fruit or vegetable surfaces are soil inhabitants*** – and are not spoilage microbes.
- ***Spoilage microbes can be introduced***
    - on seeds
    - during harvesting
    - during storage and distribution
- ***Fruits have nutrient-rich tissues and a high internal water content (>97%)*** – great conditions for spoilage microbes!
- ***Most fruits have a natural acidity*** (pH of 3.6 to 4.3) that serves as a spoilage barrier, especially bacteria; vegetables have a higher pH, generally 5.1 to 6.0, making them susceptible to a wider array of microbe spoilers – bacteria, yeasts as well as fungi.
- ***Fruits and vegetables possess an outer protective skin, typically covered by a natural waxy cuticle layer*** (limiting microbe attachment). Further, non-spoiling microbes regularly inhabit this skin region ready to compete (and usually win) against a spoilage invader!
- ***External damage (e.g. bruising, cracks, and punctures) creates microbe entry sites for food spoilage microbes.***
- ***Fresh-cut fruits lose many of their protective properties, opening to spoiler invaders.***
- ***Film-packaged cut fruits enable bacteria to compete with other spoilers*** – often producing watery-transparent, and odorous, biofilms!

Information Table 1
Expected Fruit Spoilage Microbes (F) = FUNGI (B) = BACTERIA (Y) = YEAST

	Penicillium (F)	***Aspergillus (F)***	***Geotrichum (F)***	***Fusarium (F)***	***Mucor (F)***	***Rhizopus (F)***	***Pseudomonas (B)***	***Bacillus (B)***	***Candida (Y)***	***Rhodotorula (Y)***	***Saccharomyces (Y)***
Apples	+	+			+						
Bananas		+		+							
Berries	+				+	+					
Citrus	+		+			+					
Grapes	+										
Melons	+	+							+	+	+
Peaches	+	+									
Pears	+	+			+						
Pineapple				+							
Tomato	+				+	+	+	+			

Guided Investigation

Investigating What Microbes Spoil Fruit

Objectives:

- *Identify* the kinds of microbes that spoil fruit
- *Create* a 'Spoilage Journal'
- *Understand* the timeline and conditions for fruit spoilage

What You Need:

- TSA / RB Microslide® paddle [KIT]
- Fruit sample(s) [LOCAL]
- Box of toothpicks [LOCAL]
- Magnifier [LOCAL]
- Laboratory Notebook [LOCAL]
- Digital camera (optional) [LOCAL]w
- Supervising adult!

What You Do -11 Steps:

SAFETY: Remember to wash your hands after handling fruit samples.

1. As your teacher directs, take a trip to the produce section of your local grocery store or farmer's market.
  - **(a)** Scour the fruits for spoiled examples – take close-up photos for later comparison and identification.
  - **(b)** Purchase blemished fruits.
  - **(c)** Purchase unblemished fruits.

2. In the laboratory:
  - **(a)** Use a magnifier to closely examine the skin of an apparently unblemished fruit sample. Can you find any tiny cuts, gashes, or crushed areas? If so, place the fruit in an out-of-the-way location and observe it over a 5-10 day period for signs of spoilage.
  - **(b)** Create an intentional skin injury by using a toothpick to puncture the fruit sample's skin in one or two places (or use a knife to slice up the fruit). Set these samples in an out-of-the-way location and observe it over a 5-10 day period for signs of spoilage.
  - **(c)** Use the *Fruit Spoilage Micro-Community Guide*, and a magnifier, to identify visible microbe colonies.

3. Observe all fruit samples. Use a digital camera to photograph interesting skin blemishes, and/or microbe growth!

4. Create a 'Food Spoilage Journal' record of observations in your Laboratory Notebook.

5. Use the *Information Table 1* as a guide for what spoilage microbes to expect.

6. To use a paddle to direct-sample growing spoilage microbes:
  - **(a)** Twist to remove the paddle from the vial.
  - **(b)** Allow the paddle surface to come into physical contact with the fruit surface. Contact both paddle surfaces on various spoilage surfaces (e.g. interior and exterior).
  - **(c)** Replace the inoculated paddle into the vial.

7. Secure the paddle with transparent sticky tape. Wash your hands!

8. Incubate the paddle for 5-7 days at room temperature away from sunlight.

9. Monitor daily for signs of colony growth – DO NOT ATTEMPT TO REMOVE THE PADDLE FROM THE VIAL. Instead, make all observations *through* the vial. Use a magnifier to help you observe finer details of your microbe finds!

10. Use the *Fruit Spoilage Micro-Community Guide* and the Counting Panels to presumptively (tentatively) identify fruit microbes. Record this data in your Laboratory Notebook.

11. If you have a digital camera (iPad or iPhone, or similar device), take close-up images of microbe colonies growing on fruits to color print for later review and identification.

DATA TABLE 1
Microbe Spoilage Journal

Date	*Condition*	*Observations*	*Notes*

Spoiled Fruit Samples:
Practice identifying the spoilage microbes in these samples:

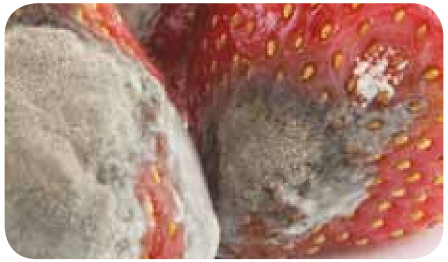


Figure 2 - Spoiled strawberries.
Source: Kenneth G. Rainis

Figure 3 - Cut tomato.
Source: Kenneth G. Rainis

Figure 4 - Spoiling lemons.
Source: Kenneth G. Rainis

Figure 5 - Spoiling apple.
Source: Kenneth G. Rainis

Designing Experiments

Remember that an experiment is a process or study that results in data (information). The results of experiments are not known in advance. Experimental design is a process of planning a study (experiment) to meet specified objectives.

Follow the scientific method – a process involving:

- Formulating a question
- Making an educated guess (a hypothesis)
- Testing your guess (experimenting)
- Analyzing your results
- Drawing conclusions (gaining knowledge; being able to explain)

More Ideas! (open enquiry)

1: Microbe Race! Locate a piece of moldy fruit. If you can, identify the microbe. Wet a toothpick and move it around the mold area so that you pick up mold spores. Select identical, but unblemished fruits, inoculate each fruit by stabbing it with the inoculated end of the toothpick. Place the inoculated fruits at various temperatures. How does temperature affect fruit spoilage?

2: Microbe Race 2! Locate a piece of moldy fruit. If you can, identify the microbe. Wet a toothpick and move it around the mold area so that you pick up mold spores. Select different fruits, inoculate each fruit type by stabbing it with the inoculated end of the toothpick. Place the inoculated fruits at various temperatures. Do all the fruits spoil? Why not?

3: Microbe Race 3 - Are cut fruits more susceptible to microbial attack (spoilage) than uncut fruits?

4: Microbe Race 4 - Time Lapse! Locate a piece of moldy fruit. If you can, identify the microbe. Wet a toothpick and move it around the mold area so that you pick up mold spores. Use a time-lapse app (e.g. *Lapse It* in the iTunes store) to visually record what occurs over a 1-2 day period.

5: Design an investigation that samples microbes from the skins of unblemished fruits like grapes. What kinds of microbes normally are found?

Once you have investigated some of the conditions under which fruit spoiling microbes act, answer these questions:

1. What is microbe spoilage and what conditions cause it?

2. What kinds and types of microbes are fruit spoilers?

3. List the conditions most likely to cause fruit spoilage (in order of severity):

4. What kinds of microbes would you expect to find on spoiled fruits?

5. Develop a chronology (timeline) that describes how a fruit spoils.

For more information:

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