How Pasteurization Affects Bacterial Growth

Louis Pasteur (1822–1895)

Topic

Heating raw milk kills the majority of bacteria present.

Introduction

In the 19th century, food spoilage was a serious problem. Refrigeration, which slows food spoilage, was not developed until the early 1800s, and home refrigeration did not become commonplace until late in the 19th century. Louis Pasteur discovered a way to prevent spoilage when working with the wine industry. He found that applying heat just below the liquid's boiling point would destroy the spoiling agents. The process, pasteurization, was named in his honor. Later *pasteurization* was applied to raw milk. In this experiment, you will pasteurize a sample of raw milk.

Time Required

50 minutes on day 1
30 minutes on day 2
10 minutes each on days 3 through 5

Materials

- 100 milliliters (ml) of raw milk
- 100-ml beaker
- 500-ml beaker

- hot plate
- sterile pipettes
- 2 prepared sterile agar petri dish
- 2 sterile bacterial spreaders
- incubator with thermometer (Celsius)
- additional thermometer (Celsius)
- two sheets of butcher (bulletin board) paper about 2 feet (ft) (60.9 centimeter [cm]) square
- sterile gloves
- face mask
- goggles
- lab apron
- alcohol spray bottle
- marking pen
- paper towels
- potholders
- 2 medium-sized sterile test tubes with caps
- beaker tongs
- test tube rack
- refrigerator
- science notebook

Safety Note **Please review and follow the safety guidelines. Wear gloves, goggles, apron, and face mask when working with food products. Wash your hands thoroughly after working with any food specimen.**

Procedure: Day 1

1. Put on all safety gear except the gloves. Wash your hands with the antibacterial soap and dry on paper towels. Put your sterile gloves on.

2. Prepare your work area by spraying it with the alcohol. Dry everything with paper towels.
3. Place a square of butcher paper on your work area and spray lightly with alcohol.
4. Place all materials to be used on top of the paper except for the hot plate.
5. Pour 50 ml of raw milk into the 100-ml beaker.
6. Insert the beaker of milk into the 500-ml beaker.
7. Pour enough water into the 500-ml beaker to reach halfway up the smaller beaker. *Be sure not to get any water into the beaker of milk* (see Figure 1). Use beaker tongs to hold the beaker of milk upright in the beaker of water.

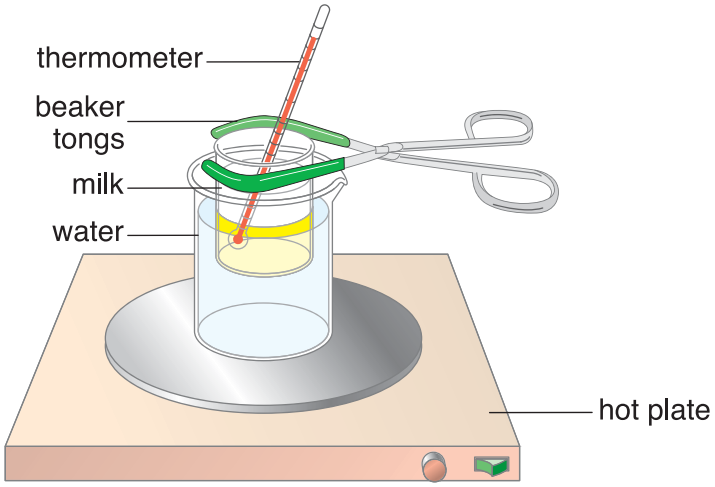

Figure 1

8. Carefully place the beakers on the hot plate, then turn it on. Allow the water to come to a boil.
9. Use the thermometer to check the temperature of the milk. Continue to heat until the milk reaches 63° Celsius (C).
10. Turn the hot plate down and adjust it so that the milk stays at or about 63°C. Keep the milk at this temperature for 30 minutes. This process pasteurizes the milk.
11. Label one sterile test tube as “Exp” and the other as “Cont.” Place the test tubes in the test tube rack.
12. Turn off the hot plate and remove the beakers, using the potholders.

13. Using the tongs, carefully remove the smaller beaker.
14. Use a sterile pipette to transfer 3 ml of milk from the beaker to the Exp test tube.
15. Use another sterile pipette to transfer 3 ml of unheated raw milk to the Cont test tube.
16. Place the rack with both test tubes in the refrigerator until day 2.

Procedure: Day 2

1. Put on all safety gear except the gloves. Wash your hands with the antibacterial soap and dry on paper towels. Put on your sterile gloves.
2. Prepare your work area by spraying it well with the alcohol. Dry everything with paper towels.
3. Place a square of butcher paper on your work area and spray it lightly with alcohol.
4. Use the marking pen to label the two petri dishes. On one petri dish, write Exp on the side of the lid and on the bottom. On the other dish, write Cont on the side of the lid and on the bottom. Set both dishes on top of the butcher paper.
5. Turn on the incubator and set it for 30°C. Write the temperature in your science notebook.
6. Remove the rack and tubes form the refrigerator and place them on the white paper.
7. Use a sterile pipette to transfer 1 ml of milk from the Exp tube to the Exp petri dish.
8. Spread the solution gently over the agar using a sterile spreader. Cover it with the lid and place it in the incubator.
9. Repeat Steps 7 and 8 for the Cont tube and petri dish. Allow both dishes to remain undisturbed until day 3.

Procedure: Days 3–5

1. Put on all safety gear except the gloves. Wash your hands with the antibacterial soap and dry on paper towels. Put on your sterile gloves.

2. Remove both petri dishes from the incubator. Observe the agar for any signs of bacteria growth such as opaque spots or a thin layer of transparent material on top of the agar. In your science notebook, create a data table to record your findings. Include the temperature you recorded in step 5 on day 2, and describe any signs of growth.
3. Repeat steps 1 and 2 on days 4 and 5.
4. Clean up materials according to your teacher's instructions.

Analysis

1. Why were both test tubes placed in the refrigerator on day 1?
2. Why was the raw cow's milk heated for 30 minutes?
3. In which petri dish did bacterial growth occur? Why did this happen?
4. In which petri dish was there no bacterial growth? Why?
5. How does pasteurization effect the growth of bacteria in milk?

What's Going On?

Milk spoils when bacteria grow in it. There are two types of bacteria found in milk: lactic acid bacteria and coliforms. Lactic acid bacteria are often cultivated for the production of cheese and yogurt. Coliforms are the primary culprits in spoiled milk. Bacteria are not the only problems in milk preservation. Some of the normal enzymes in milk can cause changes that make it undesirable. Heating destroys active enzymes as well as bacteria. After heating, milk is sealed in containers that protect it from contamination.

Want to Know More?

See Our Findings.

OUR FINDINGS

HOW PASTEURIZATION AFFECTS BACTERIAL GROWTH

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Suggestion for class discussion: Some people prefer to drink unpasteurized milk and apple juice. Ask students if they see any danger in this practice. Point out that some people have been infected with *E. coli* from drinking unpasteurized apple juice.

Teacher notes: If raw milk is not available, you can substitute raw (unpasteurized) apple juice, which can be purchased in the organic food section of the grocery store. Face masks can be purchased at home improvement stores or from science supply houses such as Carolina Biological Supply. Prepare a 10 percent bleach bath of commercial bleach and water for disposing of bacterial cultures.

Analysis

1. To keep the temperature constant.
2. To destroy any bacteria and spoilage enzymes within the sample.
3. The Cont plate. Since the milk was not pasteurized, any bacteria present were able to reproduce. The spoilage induced by the enzyme present assisted the process.
4. The Exp plate. Since the sample was heated, any bacteria and spoilage enzymes were destroyed and denatured.
5. Pasteurization stops or slows growth of bacteria.

SAFETY PRECAUTIONS

Review Before Starting Any Experiment

Each experiment includes special safety precautions that are relevant to that particular project. These do not include all the basic safety precautions that are necessary whenever you are working on a scientific experiment. For this reason, it is absolutely necessary that you read and remain mindful of the General Safety Precautions that follow this note. Experimental science can be dangerous, and good laboratory procedure always includes following basic safety rules. Things can happen very quickly while you are performing an experiment. Materials can spill, break, or even catch fire. There will be no time after the fact to protect yourself. Always prepare for unexpected dangers by following the basic safety guidelines during the entire experiment, whether or not something seems dangerous to you at a given moment.

We have been quite sparing in prescribing safety precautions for the individual experiments. For one reason, we want you to take very seriously every safety precaution that is printed in this book. If you see it written here, you can be sure that it is here because it is absolutely critical.

Read the safety precautions here and at the beginning of each experiment before performing each lab activity. It is difficult to remember a long set of general rules. By rereading these general precautions every time you set up an experiment, you will be reminding yourself that lab safety is critically important. In addition, use your good judgment and pay close attention when performing potentially dangerous procedures. Just because the book does not say “Be careful with hot liquids” or “Don’t cut yourself with a knife” does not mean that you can be careless when boiling water or using knives. Notes in the text are special precautions to which you must pay special attention.

GENERAL SAFETY PRECAUTIONS

Accidents caused by carelessness, haste, insufficient knowledge, or taking an unnecessary risk can be avoided by practicing safety procedures and being alert while conducting experiments. Be sure to check the individual experiments in this book for additional safety regulations and adult supervision requirements. Anytime you are working with an electrical current, it becomes possible to shock yourself on exposed wires. If you will be working in a lab, do not work alone. When

you are working off-site, keep in groups with a minimum of three students per group, and follow school rules and state legal requirements for the number of supervisors required. Ask an adult supervisor with basic training in first aid to carry a small first-aid kit. Make sure everyone knows where this person will be during the experiment.

PREPARING

- Clear all surfaces before beginning experiments.
- Read the instructions before you start.
- Know the hazards of the experiments and anticipate dangers.

PROTECTING YOURSELF

- Follow the directions step by step.
- Do only one experiment at a time.
- Locate exits, fire blanket and extinguisher, master gas and electricity shut-offs, eyewash, and first-aid kit.
- Make sure there is adequate ventilation.
- Do not horseplay.
- Keep floor and workspace neat, clean, and dry.
- Clean up spills immediately.
- If glassware breaks, do not clean it up by yourself; ask for teacher assistance.
- Tie back long hair.
- Never eat, drink, or smoke in the laboratory or workspace.
- Do not eat or drink any substances tested unless expressly permitted to do so by a knowledgeable adult.

USING EQUIPMENT WITH CARE

- Set up apparatus far from the edge of the desk.
- Use knives or other sharp, pointed instruments with care.
- Pull plugs, not cards, when removing electrical plugs.
- Clean glassware before and after use.
- Check glassware for scratches, cracks, and sharp edges.
- Let your teacher know about broken glassware immediately.
- Do no use reflected sunlight to illuminate your microscope.
- Do not touch metal conductors.
- Use alcohol-filled thermometers, not mercury-filled thermometers.

USING CHEMICALS

- Never taste or inhale chemicals
- Label all bottles and apparatus containing chemicals
- Read labels carefully.
- Avoid chemical contact with skin and eyes (wear safety glasses, lab apron, and gloves).
- Do not touch chemical solutions.
- Wash hands before and after using solutions.
- Wipe up spills thoroughly.

HEATING SUBSTANCES

- Wear safety glasses, apron, and gloves when boiling water.
- Keep your face away from test tubes and beakers.
- Use test tubes, beakers, and other glassware made of Pyrex™ glass.
- Never leave apparatus unattended.
- Use safety tongs and heat-resistant gloves.
- If your laboratory does not have heat-proof workbenches, put your Bunsen burner on a heat-proof mat before lighting it.
- Take care when lighting your Bunsen burner; light it with the airhole closed, and use a Bunsen burner lighter rather than wooden matches.
- Turn off hot plates, Bunsen burners, and gas when you are done.
- Keep flammable substances away from flames and other sources of heat.
- Have a fire extinguisher on hand.

FINISHING UP

- Thoroughly clean your work area and any glassware used.
- Wash your hands.
- Be careful not to return chemicals or contaminated reagents to the wrong containers.
- Do not dispose of materials in the sink unless instructed to do so.
- Clean up all residues and put in proper containers for disposal.
- Dispose of all chemicals according to all local, state, and federal laws.

BE SAFETY CONSCIOUS AT ALL TIMES!