EXPERIMENT 5 VISUAL AND MICROSCOPIC EXAMINATION OF RAW AND PROCESSED PRODUCT

Structure

5.1 INTRODUCTION

Isolation and identification of microbial food contaminants help to understand how infectious agents enter and spread through the food chain. There is a need to estimate the risk that food borne pathogens pose to human health in a national and international context and to identify possible interventions to reduce or eliminate these risks.

Objectives

After studying and performing this experiment, you should be able to:

- take visual observations of food samples
- visualize food-borne micro-organisms under the microscope.

5.2 EXPERIMENT

5.2.1 Principle

The potential for food to become contaminated with chemical substances or microorganisms starts from the time it is harvested and continues right through until the time it is eaten. The examination of food samples is one of the most important tasks:

Initial record of specimen as received

- Examine the specimen carefully for information such as to how it was received, condition (frozen, fresh), time, date, mode of delivery and write description of the specimen immediately.
- Examine seals for faults or damage and describe and note the details on the label.
- Weigh/ Measure the specimen as received and prior to opening.

Odour and taste

- Smell the food. The odour of a food can give clues to the nature of the complaint (volatile substances, deterioration, chemical taints etc.) Food may be required to be tasted CAREFULLY at this point.

Spoilage

- Visually observe spoilage by turbidity, gas production, bubbling etc.

Mouldy food

- Document a full description of the affected areas recording the types of colonies present, their colours and their textures. Measure the area(s) of suspect mould as soon as possible and in three dimensions if applicable. Ensure that the dimensions and numbers of individual colonies are noted.

Microscopic Examination of Foods

Principle

Microscopes are instruments that are capable of producing a magnified image of a small objects including microorganisms. In a food microbiology laboratory Compound Microscopes are most commonly used. These microscopes are light illuminated. They are used in observation and description of the microscopic morphology of bacteria, fungi, parasites and host cells in various stained and unstained preparations.

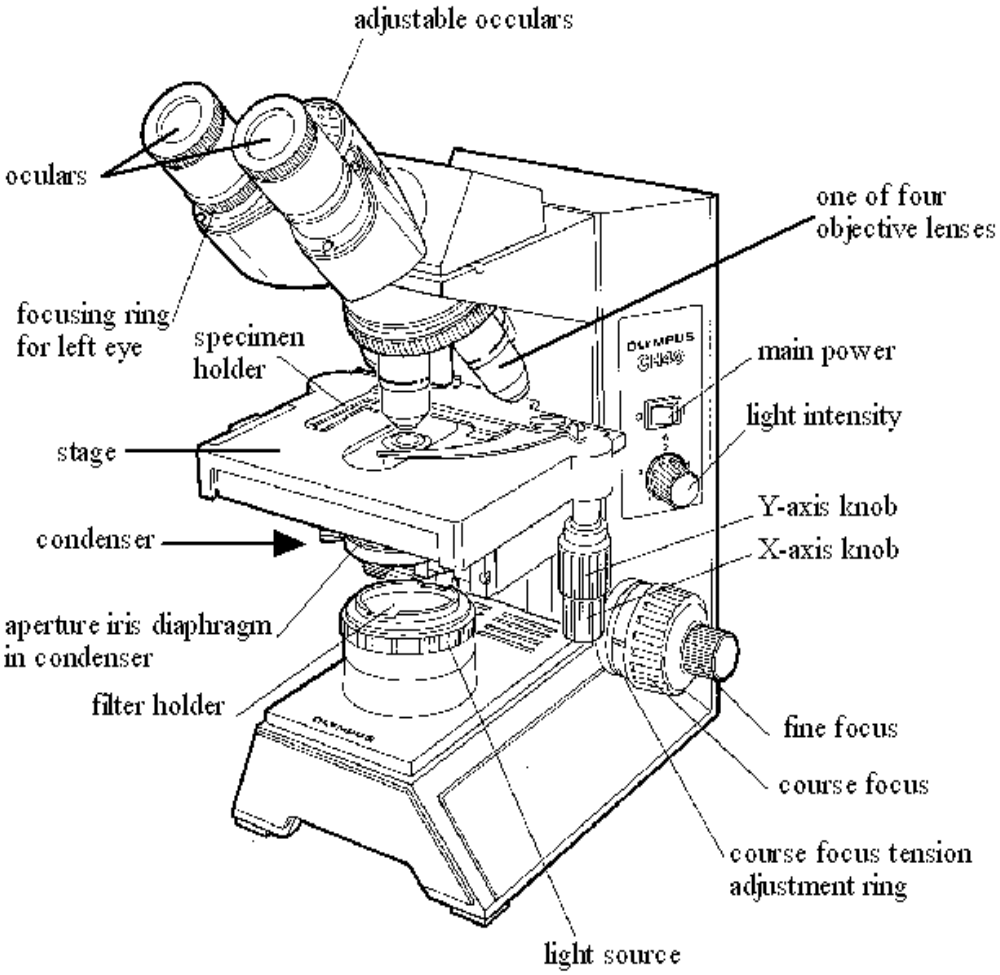


Figure 5.1: Labelled diagram of a compound microscope

Procedure to Focus the Specimen in Microscope

1. Obtain a prepared slide of contaminated food/ isolated microorganism. Mount the slide onto the stage of the microscope.
2. Start with the lowest power objective in place. Using the course adjustment knob, move the objective lens to its lowest point. Look through the ocular and focus upward with the coarse adjustment until an image comes into view. Use the fine adjustment to obtain maximum clarity. From this point on, do not use the coarse adjustment; doing so can result in damage to the lens, slide or both. Adjust the iris to allow enough light for maximum visibility and contrast. Usually, this will be about half the maximum iris opening. Too much light can wash out the details of the image.
3. Move the slide to a point of interest. Move the next objective lens into place and adjust the fine focusing knob, and adjust the iris as necessary. Repeat this step with the highest power, non-oil lens.
4. Note that as the power of the objective lens increases, the distance between the objective and the specimen (working distance) decreases. Also, as magnification increases, the field of view (visible area) and depth of field/focus (visible thickness) decrease. Moving the fine adjustment up and down allows viewing of other areas along the depth of thickness of the specimen).
5. To use the oil-immersion lens, move the turret halfway between the high-power air (non-oil) lens and the oil lens. Place a drop of immersion oil directly on the slide. Move the oil-immersion lens into place and adjust the fine focusing knob. Adjust the iris as necessary. Make sure that the immersion oil does not get on the air lenses. Make note of the differences and similarities between the organisms.
6. After using the oil lens for a specimen, wipe the lens with a piece of lens paper. Do not use anything but lens paper to clean microscope lenses. Usually, lens-cleaning fluids are not necessary unless the lens is exceptionally dirty.

For Getting the Best Possible Image

1. Use lens tissue, to clean the ocular and objective lenses; do not use any other kind of paper. You may also need to clean the slide.
2. Always begin to focus the microscope with the low power, coarse focusing knob.
3. For best viewing at high power, white light is essential. The higher the power of the objective lens, the less will be the depth of field.

Microorganism's Morphology Using the Microscope

Moulds

Mould mycelium and spores can be observed in unstained wet mounts at magnifications of x100 although direct observations of "mouldy" material through the lid of a Petri dish or specimen jar at lower magnifications with the plate microscope are also informative (but keep the lid on!). Routine

identification of moulds is based entirely on the appearance of colonies to the naked eye and of the mycelium and spores in microscopical preparations.

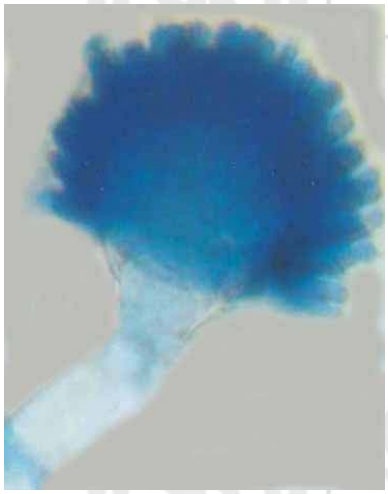


Figure 5.2: *Aspergillus sp.* under microscope

Yeast

Yeast can be seen in unstained wet mounts at magnifications ×100.

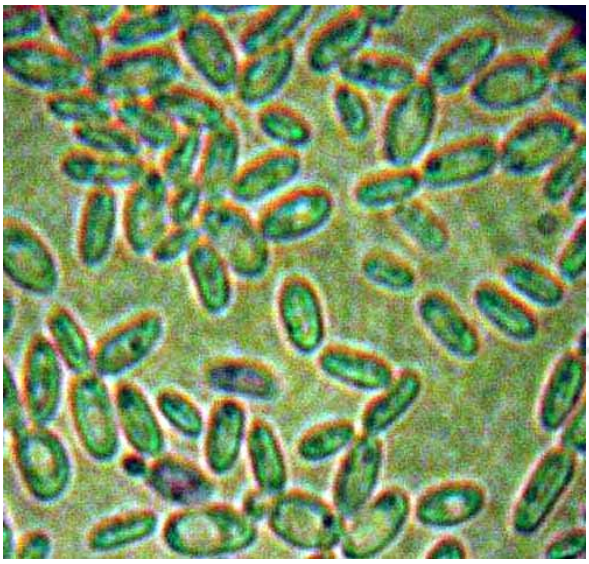


Figure 5.3: Yeast under microscope

Bacteria

Bacteria are much smaller and can be seen unstained at ×400 but only if the microscope is properly set up and all that is of interest is whether or not they are motile. A magnification of ×1000 and the use of an oil immersion objective lens for observing stained preparations are necessary for seeing their characteristic shapes and arrangements. If there is doubt that a food has caused food poisoning or has undergone microbial spoilage, the original product or a low serial dilution of it should be used to prepare a slide for direct microscopic examination. The Gram stain reaction and cellular morphology of the bacteria on the slide may indicate the need for other types of examination. A microscopic examination must be made, even though the food may have undergone heat treatment and the microorganisms involved may no longer be viable.

5.2.2 Requirements

1. Glass slides, 25 x 75 mm, with etched portion for labelling; 1 slide for each blended food sample (10^{-1} dilution)
2. Wire loop, 3-4 mm, platinum-iridium or nichrome, gauge No. 24 or 26
3. Gram stain reagents

Hucker's crystal violet

Solution A

Crystal violet (90% dye content) 2 g

Ethanol, 95% 20 ml

Solution B

Ammonium oxalate 0.8 g

Distilled water 80 ml

Mix solutions A and B. Store 24 h and filter through coarse filter paper.

Gram's iodine

Iodine 1 g

Potassium iodide (KI) 2 g

Distilled water 300 ml

Hucker's counterstain (stock solution)

Safranine O (certified) 2.5

Ethanol, 95% 100 ml

Working solution: Add 10 ml stock solution to 90 ml distilled water.

1. Compound Microscope, with oil immersion objective lens (95-100X) and 10X ocular
2. Immersion oil
3. Methanol
4. Xylene

5.2.3 Procedure

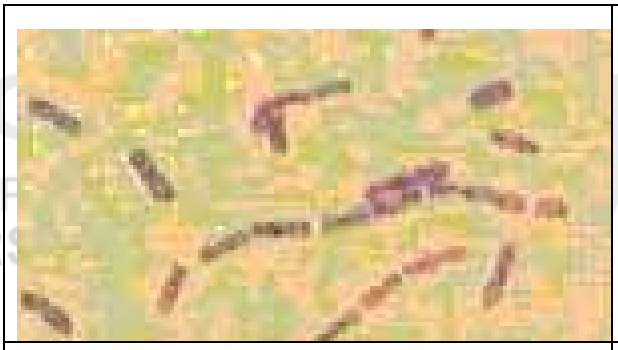
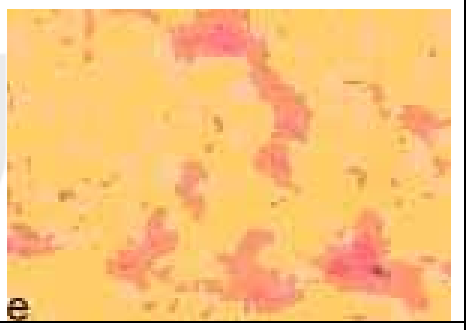
1. Prepare film of blended food sample (10^{-1} dilution).
2. Air-dry films and fix with moderate heat by passing films rapidly over Bunsen or Fisher burner flame 3 or 4 times. Alternatively, air-dry films and fix with methanol 1-2 min, drain excess methanol and flame or air-dry (this is particularly helpful for foods with a high sugar content).
3. Cool to room temperature before staining.
4. De-fat films of food with high fat content by immersing films in xylene 1-2 min; then drain, wash in methanol, drain, and dry.
5. Stain film by Gram-staining procedure.

Procedure for gram staining

1. Fix air-dried films of food sample in moderate heat. Stain films 1 min with crystal violet-ammonium oxalate solution.
2. Wash briefly in tap water and drain. Apply Gram's iodine for 1 min. Wash in tap water and drain.
3. Decolorize with 95% ethanol until blue color is no longer released (about 30 s). Alternatively, flood slides with ethanol, pour off immediately, and reflood with ethanol for 10 s.
4. Wash briefly with water, drain, and apply Hucker's counterstain (safranine solution) for 10-30 sec. Wash briefly with water, drain, blot or air-dry, and examine.
5. Examine under oil immersion and 10X ocular; adjust lighting systems to Koehlor illumination.
6. Examine at least 10 fields of each film, noting predominant types of organisms, especially clostridial forms, Gram-positive cocci, and Gram-negative bacilli.

5.2.4 Observations

- Observe the bacteria under the microscope.

	
Gram positive bacterium	Gram negative bacterium

- Record the Results in table given below.

Table 1: Gram characteristic, size, shape of two bacterial as determined following Gram staining and observation using a compound microscope

Bacterium	Gram Reaction	Cell Size (µm)	Cell Shape
Unknown 1			
Unknown 2			

5.2.5 Results

- Large numbers of Gram-positive cocci on the slide may indicate the presence of staphylococcal enterotoxin, which is not destroyed by the heat treatments that destroy enterotoxigenic *Staphylococcus aureus* strains.

- Large numbers of sporeforming, Gram-positive rods in a frozen food specimen may indicate the presence of *Clostridium perfringens*, an organism that is sensitive to low temperatures. Other Gram-positive, sporeforming rods such as *Clostridium botulinum* or *Bacillus cereus* may also be present in the food.
- When the microscopic examination of suspect food discloses the presence of many Gram-negative rods, consider the symptoms and incubation periods reported for the illness under investigation and select the specific examination method for isolating one or more of the following genera: *Salmonella*, *Shigella*, *Escherichia*, *Yersinia*, *Vibrio*, or *Campylobacter*.