IFT Experiments in Food Science Series

Microbiology in Food Systems

Activity #4

Desirable Microbial Growth in Foods: Pickle Fermentation

A Science Unit for Secondary School Curriculum

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Institute of Food Technologists
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STUDENT ACTIVITY GUIDE

Desirable Microbial Growth in Foods: Pickle Fermentation

ACTIVITY OBJECTIVE

In this activity, you will learn how the modification of one or more factors would affect the pickle fermentation and therefore the quality of the finished product.

BACKGROUND INFORMATION

This experiment introduces the basic principles associated with pickling, an important food preservation and processing technique. Many vegetables are pickled. The lactic acid–producing bacteria used to ferment olives and pickles are salt tolerant, which allows them to ferment the product in brine. This experiment will focus on the production of a common food, pickles, that demonstrates the effect that salt and other factors can have on both beneficial and spoilage microorganisms.

In this experiment, pickles will be produced by the brine method by submerging cucumbers in brine and covering with plastic film. The salt helps to withdraw nutrients (sugars) from the cucumbers. The lactic acid bacteria that are naturally present on the cucumbers are tolerant of the salt and can ferment the extracted sugars and nutrients to a variety of end products, such as lactic acid, acetic acid, carbon dioxide, and lesser amounts of other components. These end products give pickles their characteristic flavor. The bacteria that predominate during the fermentation are altered by several factors that are the topic of this experiment.

Although other species of microorganisms are present on the cucumbers, the combination of added salt and rapid production of acid early in the fermentation serves to inhibit their growth in the brine. Common spoilage microorganisms generally observed during the ongoing pickle fermentation are molds and surface yeasts. They exhibit two characteristics: (1) they are aerobic and grow on the brine surface, and (2) they are tolerant of the acid and salt levels present during the pickle fermentation. Because they must have oxygen to grow, they can usually be controlled by covering the surface of the fermentation container with a plastic sheet. Lactic acid bacteria do not require oxygen and therefore continue to grow and ferment the cucumbers.

MATERIALS REQUIRED

Approximately 30 small, whole, *unwaxed* fresh cucumbers, 7.5–15 cm long
Noncorrosive 5-liter containers, such as plastic pails or crocks.
Noncorrosive weights, such as stainless steel, bricks, stones, plastic, or wood, to keep the cucumbers submerged in the containers of brine (see diagram). Iron, steel, and copper will darken the

cucumbers.
Lightweight plastic film (such as 2-mil-thick painter's drop cloths available in hardware stores) for covering the brine surface to exclude air.
Salt (NaCl), not iodized.
Bacteriological stains: Crystal violet, Gram's iodine, Safranin (counterstain). A simple stain such as methylene blue may be used as an alternative.
pH meter or pH paper (pH range 3.0–11.0).
1,000× microscope with oil immersion lens.
50-mL burette
20-mL pipettes
200-mL beakers
1% phenolphthalein indicator
500 mL of 0.1 N NaOH
Glass slides and cover slips for staining

EXPERIMENTAL PROCEDURE

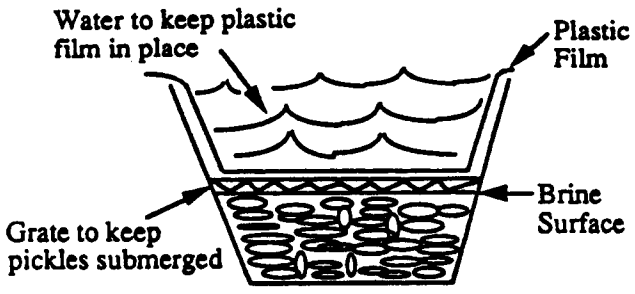
Preparation of Pickles

1. Prepare brine solutions by dissolving the following amounts of salt in 5 L of water:
   0%: 0 g
   1.5%: 75 g
   2.5%: 125 g
   10%: 500 g
   15%: 750 g

2. Add the cucumbers to the brine at a ratio of 1:1 on a weight/weight basis, as follows: Fill the plastic pail or crock 1/2 to 2/3 full of cucumbers (about 5–6 cucumbers) and add the brine.

3. Submerge the cucumbers in the brine by placing a grate over them and weighting it down with noncorrosive weights (Figure 1). A simple grate can be made by drilling holes in a Plexiglas disk. Since some foaming and frothing can be expected to occur during the first week of fermentation, an experimental option that is now a normal part of commercial production is to cover the surface with several layers of thin plastic film (such as painter's drop cloths) and place the grate and several inches of water or brine on top of the film to hold it in place and prevent growth of mold on the brine surface. In

Figure 1—setup for Pickle Fermentation



this case, first place the plastic over the pickles and the the grate. Pour water or brine over the plastic to a level which will hold the plastic down to keep air out.

4. Leave the pickles in the brine at room temperature and observe periodically for several weeks.

5. Measure the pH and acidity of the brine every two days and record the results in Table 1. To maintain anaerobic conditions when removing samples, quickly pull back a portion of the plastic and quickly remove a small amount of brine from the top of the solution, then replace the plastic as soon as possible. Changes in pH and total acidity are important indicators of the progress of the fermentation. Total acidity measures the total amount of acid that is in the solution, whereas pH measures the hydrogen ion concentration of the solution, which is related to how strong the acid concentration is. Measure the total acidity (also referred to as titratable acidity) by either titration or use of a pH meter, as described below:

Titration Method

In this method, brine is titrated with phenolphthalein until the endpoint (a faint pink color which remains permanent for 15 sec) is reached.

Pipet 18 mL of brine into a 100-mL glass beaker. Add 0.5 mL of a 1% phenolphthalein solution (1–2 drops). Titrate with 0.1 N NaOH until the endpoint is reached. Record the number of mL of 0.1 N NaOH required. Then calculate the total acidity (as acetic acid) as follows:

$$\% \text{ acidity} = \frac{(\text{mL of NaOH}) \times 0.009}{\text{sample weight in g}} \times 100$$

Since we use an 18-g sample, this can be simplified to:

$$\% \text{ acidity} = (\text{mL of NaOH}) \div 20$$

pH Method

In this method, pH is measured using a pH meter, although pH paper (pH range 3.0–6.0) may also be used.

Standardize pH meter using pH 7 and pH 11 buffer standards. Place 50 mL of brine in a 200-mL beaker. Place a stirring bar gently into the beaker and place it on an automatic stirrer. Titrate with 0.1 N NaOH to pH 8.1 using the pH meter. Record data as mL of 0.1 N NaOH added. Calculate the total acidity using the equation used in the titration method.

Microbiological Analysis

Make visual and microbial observations every five days and record the results in Table 2. Follow the

presence and succession of various types of microorganisms (molds, yeast, spherical bacteria in chains, rod-shaped bacteria, rod-shaped bacteria in chains, etc.) by taking samples of the brine, staining them, and viewing the Gram-stained or simple-stained samples under the microscope, as follows:

Preparation of Smears

Pure cultures of bacteria can ordinarily be prepared for staining by the simple process of making an aqueous suspension and drying a drop of it on a slide or cover glass, without any fixative other than gentle heat. Use of this simple procedure depends on the fact that most bacteria, because of their small size or their stiff walls, can be dried without great distortion.

To prepare a bacterial smear, remove a small amount of surface growth from the brine and mix it with distilled water. The suspension used should always be sufficiently dilute. Ordinarily, only a faint turbidity should be visible to the naked eye. If a smear after staining does not show any portions where the bacteria are well separated, a new, more dilute smear should be made. This is particularly important in the case of the Gram stain or flagella staining.

The usual method of fixing the suspension to the slide or cover glass is to first dry it on a hot plate, then pass it rapidly through a bunsen flame two or three times.

Staining Procedure

Bacteria can be differentiated into Gram-positive bacteria and Gram-negative bacteria by the following staining procedure:

Fix the dilute brine solution onto a clean slide as described above. Place a few drops of Crystal Violet Stain on the slide and let it stand for 60 seconds. Drain the extra stain from the slide. Place a few drops of Iodine Stain on the slide and let it stand for 60 seconds. Drain the extra stain from the slide and wash with water until no free stain appears in the wash. Gently wash with 95% alcohol until no free stain appears in the wash. Place a few drops of Safranin on the slide and let it stand for 45 seconds. Drain the extra stain from the slide and wash with water until no free stain appears in the wash. Air dry the slide and examine it under the microscope.

Identification of Bacteria

Gram-positive bacteria have the ability to retain Crystal Violet stain and will appear dark purple under the microscope. Gram-negative bacteria lose their ability to retain Crystal Violet stain when rinsed with alcohol. They are subsequently made visible by counterstaining with Safranin to a pink color.

Alternative Staining Method

An alternative method of staining is to stain fixed slides with methylene blue for 60 seconds, drain and rinse with water until no free stain appears in the wash, then air dry the slide and examine it under the

microscope. With this method, all cells will appear blue under the microscope, but students should be able to differentiate cells by their shape, arrangement (clumps, chains, etc.), and number.

Caution: Do not eat the pickles you produced in this experiment, because control of or elimination of pathogenic bacteria cannot be guaranteed by these processing techniques.

QUESTIONS

1. Do the same types of microorganisms appear (predominate) in all containers?

2. Are some groups present only in one container? Why or why not?

3. Were you able to absolutely exclude the growth of microorganisms using very high levels of salt? Why or why not?

4. Why are fermented pickles desalted before packaging for consumers?

5. What is the role of salt in food preservation?

6. How do the two methods of producing pickles—the brine process and the fresh-pack process—differ?

DATA TABLE—Pickle Fermentation

	Date:		Date:		Date:		Date:	Date:
Sample	**pH**	**Acidity**	**pH**	**Acidity**	**pH**	**Acidity**	**Microbial**	**Visual**
0% salt								
1.5% salt								
2.5% salt								
10% salt								
15% salt								