ABSTRACT
In an age-appropriate activity developed by a researcher-teacher team working together under the auspices of the American Chemical Science Coaches Program, middle school students at Greensboro Montessori School learned about the microbiological basis of fermentation, learned about the chemical changes that take place during the fermentation of milk into yogurt, applied this basic knowledge to designing and implementing experiments to test different conditions for culturing yogurt, assessed the outcomes of different culturing conditions, and developed a method for producing yogurt. This exercise includes hypothesis formulation, experimental design, and hypothesis testing and serves as an example of how empirically derived knowledge can be applied to the design of a food product.

**Key Words:** yogurt; microbiology; inquiry; fermentation.

**Introduction**
Fermentation of milk by lactic acid bacteria has been used for thousands of years as a method for naturally preserving milk. Yogurt, one product of this process, is a popular and well-known food (Fisberg & Machado, 2015). Teaching about fermentation with yogurt as the model can be used as an introduction to microbiology (Drake & McKillip, 2000), food chemistry (Zimmerman et al., 2019), and food production. Some basic principles about the bacteria behind fermentation can help students learn about bacterial functions and how they can be used for positive ends (Kiefer et al., 2018). Here, we demonstrate that fermentation can also be a useful tool for learning about principles of inquiry through yogurt production, from initial concepts to hypothesis-based experimentation to creation of a final product.

In an age-appropriate activity developed by a researcher-teacher team working together under the auspices of the American Chemical Science Coaches Program, middle school students at Greensboro Montessori School learned about the microbiological basis of fermentation, learned about the chemical changes that take place during the fermentation of milk into yogurt, applied this basic knowledge to designing and implementing experiments to test different conditions for culturing yogurt, assessed the outcomes of different culturing conditions, and developed a method for producing yogurt.

Importantly, the students were not simply handed the instructions on how to make yogurt and told to follow them. Instead, they predicted and tested the outcome of yogurt production under different conditions following the predict-observe-explain structure of hands-on learning experiences (White & Gunstone, 1992). Students examined and interpreted the resulting yogurt products against a background of the basic concepts in food chemistry and food microbiology. The focus of this activity was on solving problems to achieve a final product with a predetermined outcome, a good example of a project-based learning experience (Capraro et al., 2013). As a result of the experimentation, the students developed a “final” protocol, which was then applied in the production of yogurt. This protocol included ideal times and ideal milk types for yogurt production, which the students determined empirically.

We describe here the basic, easily incorporable activity for developing a method to produce yogurt within a school day and for learning to transform basic and empirically derived scientific knowledge into the production of a food product.

**Health & Safety**
The materials used (milk, yogurt) were simply purchased from a local supermarket and should be considered safe, but consumption of these commercial products should take place in a cafeteria. Yogurt produced in the laboratory was simply discarded after...
Part 1: Discussion & Background Information
(30 minutes)

Children tasted samples of plain, unsweetened commercial yogurt and were prompted to describe how the yogurt tasted. Creamy, bitter, and sour were adjectives offered by the students, who vehemently disagreed that plain yogurt could be considered sweet. The students were then asked to consider what yogurt was made from; all knew that the source material was milk. The students described milk as watery and slightly sweet. They were then asked to list the components of milk: water, protein, calcium, lactose, fat, and vitamins A and D. Class discussion of the nutritional aspects of each of these components was followed by the revelation that Lactobacillus or other bacteria are grown in milk to produce yogurt and that the lactose in milk is the source of energy for these bacteria.

The optimum growth temperatures of Lactobacillus bulgaricus and Streptococcus thermophilus, the two primary species used in yogurt cultures, are 40–44°C and 35–53°C, respectively (Sfakianakis & Tzia, 2014). However, for the purposes of this activity, the term Lactobacillus is used as a stand-in for both species, with a standard culturing temperature of 40°C that is suitable for both species.

In a brief lecture, students learned some facts about yogurt fermentation. For example, the name Lactobacillus combines two terms: lactis (or milk) and bacillus (or rod). The first term refers to the use of this bacteria in milk, and the second term refers to the shape of the bacteria as seen in a microscope. The bacillus shape contrasts with the coccus (ball) and spirillum (spiral) types of bacteria.

Bacteria grow and reproduce in a process known as binary fission. Binary fission of Lactobacillus bacteria takes roughly one hour at 40°C, so it is possible to calculate how many cells result if the initial count of cells is known (Wilkinson & Booth, 1986). Growing bacteria in a medium is known as culturing, and the conversion from milk to yogurt by bacteria is known as fermentation, as is the conversion of any food to another using microorganisms (e.g., grapes to wine, cabbage to sauerkraut; Board, 1983). Lactobacillus consume lactose, the sugar found in milk, and produces lactic acid and acetaldehyde, the chemicals responsible for the sour and bitter tastes, respectively (Gezginc et al., 2015). An individual bacterium can be considered a tiny lactose-consuming factory. As the lactose in milk is consumed, the sweet taste is lost.

The two primary proteins in milk are whey and casein. As lactic acid is produced, the pH (a measure of acidity or “sourness”) decreases. The lower the pH, the more acid a liquid is. The more lactic acid produced, the more acid or “sour” a liquid is. As the pH drops, via production of lactic acid, the casin “clumps” together, or aggregates (Sfakianakis & Tzia, 2014). This clumping is the process that is primarily responsible for the creamy texture of yogurt (Sfakianakis & Tzia, 2014).

To prepare it for fermentation into yogurt, the milk is first heated to 90°C for 10 minutes. This has a twofold purpose: (1) to remove all competing bacteria, so that the final product contains only the bacteria of interest; and (2) to aid in the aggregation process of the casein proteins (Sfakianakis & Tzia, 2014).

Part 2: Method Development & Implementation
(2 hours)

In part 2, the instructor asked the students if they would like to make yogurt and received enthusiastic positive replies. Importantly, the students were not allowed to taste the yogurt produced in the laboratory. They were prompted to use the information learned in Part 1 to design a method for culturing yogurt. They had a worksheet on which they listed the materials and methods they thought were necessary to produce yogurt, as well as a set of procedures and outcomes (Appendix).

The materials list included milk, a beaker, lactobacillus, an incubator, a hot plate, a container of iced water, a Styrofoam container, and a thermometer. The instructor gave prompts, such as when a student was missing materials or a step in the procedure: “Is it a good idea to put bacteria in liquid that is heated to 90°C? Why?” or “Should the yogurt be placed covered or uncovered in the incubator? Why?” or “Once it’s done incubating, what should we do with the culture?” (see Appendix). The students asked where they would get the Lactobacillus from and were asked to think about where they could find it, until one student suggested that they use an “old” yogurt, or the commercial yogurt. The instructor suggested that a 5% weight/volume of yogurt to milk contained sufficient amounts of Lactobacillus to ferment the milk into yogurt.

With help from the instructor, the students collectively constructed the following tentative procedure:

1. Heat 500 mL of some type of milk to 90°C in a beaker on a hot plate for 10 minutes.
2. Cool to 40°C using a container of iced water.
3. Add 25 g of store-bought yogurt to the milk.
4. Cover the beaker with aluminum foil.
5. Place the beaker in an incubator at 40°C for X hours.
6. Cool the beaker overnight in a refrigerator.

The instructors rarely intervened to simply state what the procedure for the yogurt-making should be, but relied on student answers to prompts to guide the method development until the students were left with two questions that were left open to inquiry and investigation: (1) What kind of milk should be used? (2) How much time does the culturing take?

For question 1, the instructor asked them to predict what would happen if the culturing took place with each kind of milk (Appendix). One student proposed that since whole milk was fattier, the resulting yogurt would be creamier and taste better. The students were then split into groups, with each group choosing the kind of milk they would like to use (nonfat, 2%, or whole). At least one group each was encouraged to use nonfat and 2%, since all the students highly favored whole milk. An alternative approach would be to allow all groups to test all three types of milk.

To answer question 2, the students designed an experiment in which the inoculated milk would be placed in the incubator after being divided into plastic cups. The cups would be taken out at different time points and placed in the refrigerator overnight to be assessed the next day. The students suggested three time points: one, three, and five hours. The instructor then suggested an eight-hour time
point (six hours is the minimum under these conditions to achieve the
typical yogurt-like texture). The students were asked to make pre-
dictions about the yogurt, such as what would be the outcome with
different kinds of milk, how the pH would change over time, how
the creaminess would change over time, and how sour the yogurt
would be over time. At the end of the yogurt preparation, they
measured the pH of the yogurt as a stand-in for sourness and
assessed its creaminess on a scale of 1–5. Ideal culturing times were
assessed, as well as which milk type was ideal. (Students should be
couraged to prepare their own assessment tables according to
milk type on scales of creaminess and sourness, as predicted ini-
tially.) The ideal milk was determined to be whole milk, because
of the highest level of creaminess.

The culturing process was repeated on a different day, and the
students found their ideal incubation time to be six hours, taking into
account the duration of the school day. Therefore, the final procedure
read:

1. Heat 500 mL of whole milk to 90°C in a beaker on a hot
   plate for 10 minutes.
2. Cool to 40°C using a container of iced water.
3. Add 25 g of store-bought yogurt to the milk.
4. Cover the beaker with aluminum foil.
5. Place the beaker in an incubator at 40°C for 6 hours.
6. Cool the beaker overnight in a refrigerator.

Ultimately, applying theoretical knowledge through the process
of inquiry and investigation, the students successfully produced
yogurt in the laboratory (Figure 1).

Assessment

The student worksheets were assessed for completeness and cor-
rectness. Internal motivation was assessed. Formal and informal
assessments of student engagement suggested that this was an
effective learning experience that connected ideas behind micro-
biology, food science, and chemistry.

Conclusion

This exercise teaches many science concepts, such as the nature of
prokaryotic microbes, controlling the growth of microbes and utilizing
them for positive ends, and the link between science and society.
It includes hypothesis formulation, experimental design, and
hypothesis testing. The activity can be modified depending on time
constraints. For example, the activity can be limited to testing differ-
ent milks, leaving out the different incubation times. The learning
experience can be broken into sessions on different days, a “lecture
and planning phase” and an implementation phase. The activity
can be expanded as well, to assess growth temperatures and to assess
taste directly (sourness, bitterness) if consumable yogurt can be
safely produced. Also, it can be expanded to determine the ideal
amount of starter culture to use, and to actually produce starter cul-
ture from pure strains of yogurt-producing bacteria. In addition, this
activity could be applied in activities for older children and even col-
lege-aged students.

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Appendix: Materials for Making Yogurt (What Is Each Material Used For?)

(This should be a blank numbered list for the students)

1. 500 mL milk [Prompt: How much milk?]
2. Beaker [Prompt: Where will we put the milk?]
3. Lactobacillus [Prompts: What turns the milk into yogurt? Where do we get Lactobacillus from?]
4. Hot plate [Prompt: How do we heat the milk?]
5. Cold water [Prompts: How do we cool the milk? How do we keep the water cold?]
6. Thermometer [Prompt: How do we know the temperature of the milk?]
7. Styrofoam container [Prompt: Where do we place the cold water?]
8. Optional: sterile sample cups with covers for testing different time points [Prompt: How can we easily test different time points without disturbing the cultures in their beakers?]

Procedure for Making Yogurt (Steps)

(This should be blank for the students)

1. Heat 500 mL of some type of milk to 90°C on a hot plate for 10 minutes
   Prompts: How much milk should we use? What temperature should we heat it to? We should heat it for 10 minutes.
2. Cool to 40°C using a container of iced water.
   Prompts: Should we add the Lactobacillus after heating to 90°C? To what temperature should we lower it? Why?
3. Add 25 g of yogurt to the milk (5% w/v of milk).
   Prompts: How much yogurt shall we add? Give answer: We should add 5% in mass of the volume of milk.
4. Cover the beaker with aluminum foil.
   Prompts: Should we leave the yogurt culture of milk+yogurt exposed to air? Why or why not?
5. Place yogurt cultures in incubator at 40°C for X hours. [Ideal is 6–8 hours, but you can test a range of hours. If you want to test a range of hours, use small plastic sterile sample cups with covers, each labeled with a different time, to be removed at those times.]
   Prompt: At what temperature will the fermentation work best?
6. Cool yogurt cultures overnight in a refrigerator.
   Prompt: Once it is done incubating, where should we store the newly made yogurt? Fact: refrigeration helps the yogurt set.

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Conclusion

What is the effect of culture time on sourness? Why?
Longer culture time will lead to greater sourness. This is because as the bacteria reproduce through binary fission, more and more lactic acid is produced. The presence of acid gives a sour taste to the yogurt. The more lactic acid that is present, the sourer the final product will be.

What is the effect of the type of milk used on the texture and taste of the yogurt?
The fatter the milk, the creamier and more solid the final product is.

What is the effect of time on the pH of the yogurt? Which culture leads to the yogurt with the lowest pH?
Longer culture time will lead to a lower pH. This is because as the bacteria reproduces through binary fission, more and more lactic acid is produced. The higher the amount of acid, the lower the pH measured.

Which culture time gives the most bacteria?
A longer culture time will lead to a greater number of bacteria, as the bacteria have more time to reproduce through binary fission.

How would you change the procedure to make a yogurt you like better?
Possible answers: Change the milk to make it more or less creamy. Change the culturing time to make it less sour, or more liquid, or more sour, or more solid and creamy. Add other food items like sugar, Oreo cookies, fruit, etc.