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Preparation and Testing of Food Freshness Indicators: an Application-Oriented Learning Module Integrating Basic Concepts of Microbiology and Chemistry Laboratory

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INTRODUCTION

Preservative- and additive-free fresh food products are always preferred by consumers. These foods also retain their nutritional value better. However, they are also at risk of getting spoiled easily. Food industries are coming up with smart packaging that has a visual colorimetric indicator to detect food spoilage in the early stage (1–3). We modified previously published protocols (4–7) for the preparation of smart food labels while designing the food microbiology learning module. Our module challenged the students to test if the pH indicator dyes could be used as spoilage indicators for the locally available dairy-derived food samples.

At room temperature (25°C to 30°C in summer in India), dairy products spoil easily. With time, there is an increase in bacterial sugar catabolism and fermentation reactions that occur in the dairy-derived food sample. This gives rise to an increased amount of acidic vapors. The amount of acidic vapors that accumulate in the headspace of the container increases with increased storage time of food samples. Four different pH indicator dyes, absorbed on a solid support (paper disc), were placed in the headspace of a food container (Fig. 1A to C). Each pH indicator had a different pKa. When the change in the pH of acidic vapors in the headspace of the container takes place across the pKa value of a pH indicator, the color change is visible on the disc.

In a laboratory, one of the definitive spoilage indicators is the total number of bacteria growing in the food. The colony forming unit (CFU) per milliliter is a unit commonly used to estimate the concentration of microorganisms in a test sample. Different food samples have a different threshold number of CFU per milliliter considered indicative of spoilage. The students correlate the CFU counts at 6 h, after 24 h, and after 48 h with observed color changes in four indicators to comment on the best spoilage indicator among the four dyes tested.

Learning goals

The Biology Cell of our institute conducted two 5-day camps for first- and second-year undergraduate students in March and April 2022. Approximately 20 students from different regions and institutes or colleges in India participated in each of the camps. Most of them had little or no hands-on laboratory exposure because of the COVID-imposed restrictions in the previous 2 years. Keeping these limitations in mind, the learning module was developed as a 4-day structured inquiry (8, 9). However, the structured approach can be reduced and shifted to a guided inquiry format if the students are already familiar with the laboratory techniques and if there is no limitation of time.

The execution of the module involves understanding concepts of serial dilutions, microbial growth kinetics, microbial metabolism, and pH as well as pKa. The students also learn to grow microbial cultures from food samples, spread plating (counting the total number of aerobic microbes), Gram staining, and other aseptic techniques in the process. Repeated CFU counts allow them to practice their technical skills. Meticulous annotations of color changes in the four dyes with increased incubation time and CFU counts hone observation skills in students. Additionally, the module helps students realize that the solution to the everyday problem of food spoilage detection can be found in an interdisciplinary approach integrating the basics of chemistry and microbiology.

PROCEDURE

We chose curd (a type of yogurt that sets in Indian households), kheer (a dessert cooked on high heat with sugar, milk,

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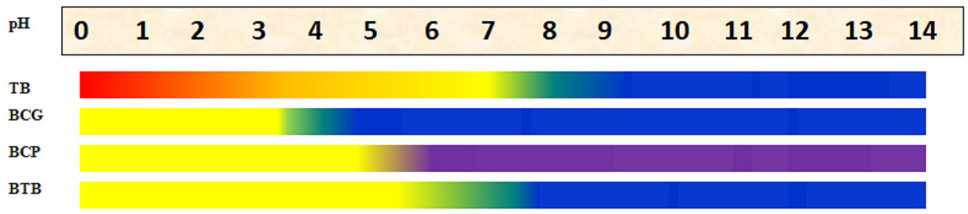
A)



B)

Indicator	Range of pH values over which the indicator changes colors from its acid form to its base form	pKa
Thymol Blue (TB)	1.2 to 2.8 and 8.0 to 9.6	1.6 ,8.9
Bromocresol green (BCG)	4.0 to 5.6	4.7
Bromocresol purple (BCP)	5.2 to 6.8	6.3
Bromothymol blue (BTB)	6.0 to 7.6	7.0

C)

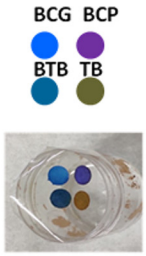


FIG 1. Schematic illustrating changes in the color of the selected pH indicator dyes with change in pH (A), their pH range and pKa values (B), and how they are placed with the help of cello tape on a small plastic container filled with water (C).

and boiled wheat), and commercial yogurt (with added preservatives and acidity regulators) as food samples. We used water as a control. All samples and water were loaded to the same level (5 mL) in small plastic containers (10 mL). As a first step, we set up pH indicator discs in the headspace of food packaging. Dylon color catcher (DCC) is a highly absorbent commercial dye catcher available for online purchase in India. Students cut DCC paper using a punching machine to prepare small discs. The pH of all of the indicator dye solutions was adjusted to approximately neutral (7.0). The discs were then dipped in pH indicator dyes for a few minutes. If very little dye is absorbed on the disc, the indicator changes color too fast in response to the generation of acidic vapors in the headspace. In contrast, too much dye makes it difficult to observe a slight change in the acidity. Other precautions included not touching paper discs or the cello tape beneath them with hands (see Fig. S1A and B in the supplemental material). The paper discs were handled with forceps and allowed to air dry on a glass slide. Next, they were stuck on cello tape. The tape was placed on a food container with the paper discs facing the food (Fig. 1C). Students noted the colors of dyes on the provided observation sheet after 0, 6, 24, and 48 h of storage at room temperature (Table 1). The second step was to set up a bacterial spread plate assay of the kheer or curd sample stored at room temperature. This step was repeated after 6, 24, and 48 h of storage (Table 1). After 24 h of incubation of nutrient agar plates at 37°C, the students counted the total number of aerobic microbial colonies. Later, they converted the total colony count into CFU per milliliter for all three plates.

On the last day, students checked if and when the number of CFU per milliliter crossed the threshold value indicative of food spoilage. We used threshold values reported in published food safety-related literature to compare the CFU per milliliter values observed in our module. In our camp, kheer spoiled after

TABLE 1
Overview of student activities carried out each day for preparation and testing of food freshness indicators[a]

Student activity	Day I	Day II	Day III	Day IV
Set up pH indicator labels	√ 0 h storage			
Observe color of indicator dyes	√ 6 h storage	√ 24 h storage	√ 48 h storage	
Inoculate spread plate	√ 6 h storage (plate I)	√ 24 h storage (plate II)	√ 48 h storage (plate III)	
Count colonies		√ For plate I	√ For plate II	√ For plate III
Concluding experiment				√ Submissions of worksheet

[a]Left most column lists student activities. The days when the specified activity is scheduled in our learning module are indicated by √.

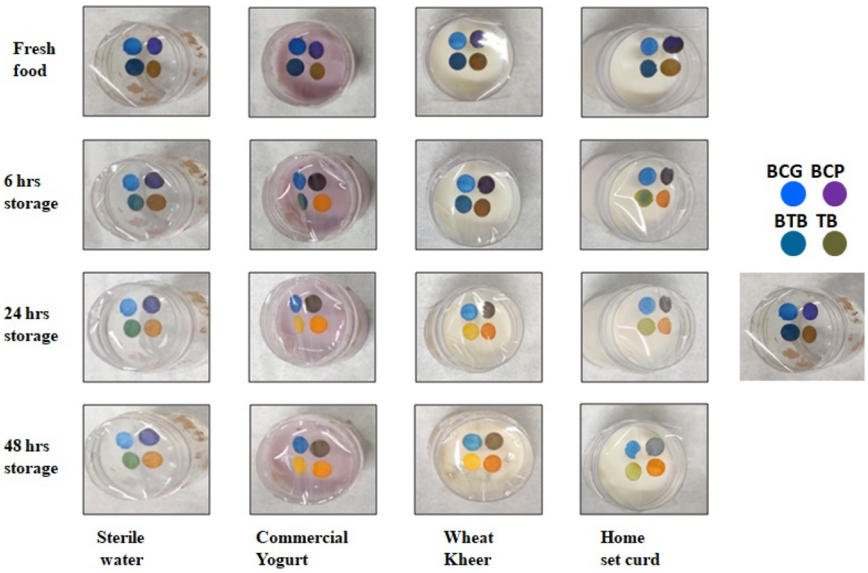


FIG 2. Representative color changes observed with increased storage time in the three samples tested. Water is used as a control. BCG, bromocresol green; BCP, bromocresol purple; BTB, bromothymol blue; TB, thymol blue.

just overnight incubation. The time needed for the curd to spoil varied with different samples tested. The boiled kheer sample at 0 h of storage was used as the negative control. The students then correlated food spoilage with color changes in the food labels (Fig. 2). Next, they commented on which of the dyes would be most and least suited to be used as a freshness indicator for the food sample tested.

Yogurt was used in the module to discuss limitations of testing methods and possible further development. Acidity in the headspace of the commercial yogurt increased, but only one or two microbial colonies grew on nutrient agar even after 48 h of storage. Possibly, the active culture in commercial yogurt did not grow well on the nutrient agar medium.

Safety issues

Many of the food spoilage-causing bacteria can be opportunistic pathogens. The microbiological work is recommended to be completed in a biosafety level 2 (BSL-2) space following ASM biosafety guidelines for teaching laboratories. Wearing safety glasses, laboratory coats, gloves, and a mask is recommended when the students handle spoiled food samples in the BSL-2 space. The students were aware of good lab practices and safety guidelines through virtual and hands-on labs, as they had completed a minimum 6 months of their coursework. However, because of COVID- imposed restrictions, we assumed them to be novices. We trained each student as they handled nonspoiled samples on the first day. The entire activity was carried out in the presence of a facilitator and laboratory assistants. We would like to stress that prior demonstrated efficiency of handling a BSL-1 microorganism is recommended before the students start working in BSL-2 space, per ASM biosafety guidelines. Potentially hazardous waste should be discarded following the issued regulatory guidelines. All instruments, equipment, and workspaces should be sterilized and sanitized before and after use, as applicable.

CONCLUSION

Our module was designed to encourage and engage first-year students in the science laboratory. We were enthused to see students' laboratory reports reflecting their sincere involvement and a good understanding of the inquiry. While designing the module, we observed that many parameters of the design can be worked upon to test potential of this activity to expand it as an elaborate research experience. Examples of variables related to the module are the choice of pH indicator dyes, tape and container to be used, storage temperature, type of food sample to be tested, choice of absorbent paper, amount and concentration of dye absorbed on the paper, choice of medium to grow the microorganisms, inclusion of other methods of assessing food spoilage. All of these variables can be standardized with iterations of the protocol. However, the activity is not yet validated for its effectiveness as a course-based undergraduate research experience, also known as a CURE (10). Finally, the module is relevant to the majority of the basic biology laboratory curricula and its execution does not require a lot of infrastructure or consumables, making it easier to incorporate into regular undergraduate courses.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.2 MB.

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