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Sato, Brian K Alam, Usman Dacanay, Samantha J et al.

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Brewing for Students: An Inquiry-Based Microbiology Lab †

Brian K. Sato¹*, Usman Alam¹, Samantha J. Dacanay¹, Amanda K. Lee¹, and Justin F. Shaffer²

¹Department of Biochemistry and Molecular Biology, University of California, Irvine, CA 92697,

²Department of Developmental and Cell Biology, University of California, Irvine, CA 92697

In an effort to improve and assess student learning, there has been a push to increase the incorporation of discovery-driven modules and those that contain real-world relevance into laboratory curricula. To further this effort, we have developed, implemented, and assessed an undergraduate microbiology laboratory experiment that requires students to use the scientific method while brewing beer. The experiment allows students to brew their own beer and characterize it based on taste, alcohol content, calorie content, pH, and standard reference method. In addition, we assessed whether students were capable of achieving the module learning objectives through a pre-/posttest, student self-evaluation, exam-embedded questions, and an associated worksheet. These objectives included describing the role of the brewing ingredients and predicting how altering the ingredients would affect the characteristics of the beer, amongst others. By completing this experimental module, students accomplished the module objectives, had greater interest in brewing, and were more likely to view beer in scientific terms.

INTRODUCTION

As biology instructors, we strive to instill in our students the curiosity and excitement that we feel when tackling a scientific problem. Unfortunately, this does not always come across, as students often view undergraduate biology education as memorization-based and lacking in the development of critical thinking skills (2, 12, 21). These, and other issues, have been highlighted in recently released national reports regarding STEM education (2, 13-15), which also include various means to address these problems. Suggested solutions include a renewed emphasis on highlighting topics that are relevant to the real world and students' daily lives, increased exposure to the scientific method, and integration of different disciplines within a single course. Education research has identified a number of interventions to address the problems presented in these reports, such as active learning (1, 5), increased course structure (6, 8), and the introduction of primary literature into the classroom (9, 17), to name a few.

Of the various biology disciplines, microbiology is arguably one of the easiest to relate to students' everyday lives. Microbes are ubiquitous in the environment and have

countless positive and negative influences on society. This relevance can be seen in the wide variety of published microbiology lab modules including those with medical (7, 20), environmental (3, 16), and food processing (19, 22) themes. A number of these cited experiments are also discovery-driven, in that they have an unknown outcome, allowing students to participate in the scientific method. These types of experiments have been shown to be beneficial for students, resulting in increased learning and interest in science (11). Despite these benefits, the creation and implementation of discovery-driven modules can be challenging, as they may require more preparation, have higher costs, and could be more difficult to scale for large-enrollment courses (18).

To this end, we have created a straightforward, easy-to-implement, discovery-driven brewing module. Beer is an especially relatable and significant topic for a microbiology course because it is a common beverage in the United States that would not exist without microbes. In this experiment, undergraduates select their ingredients for brewing, use primary literature to predict the impact of these ingredients on the final product, and characterize their beer with a variety of methods. The learning objectives for the module can be found in Table 1.

This module has been implemented in a microbiology lab at a large-enrollment research university. Using the established protocol, students were able to successfully brew their own beer and achieve the module objectives as demonstrated through a variety of assessments, including a pre-/posttest, a self-assessment, and a final exam. In addition, students were more interested in brewing and were

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^{*}Corresponding author. Mailing address: Department of Biochemistry and Molecular Biology, 2238 McGaugh Hall MC3900, University of California, Irvine, CA 92697. Phone: 949-824-0661. E-mail: bsato@uci.edu.

[†]Supplemental materials available at http://jmbe.asm.org

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TABLE 1.

Module learning objectives and methods of assessment.

Learning Objectives	Assessment Method
Describe the role of the brewing ingredients, including malt extract, yeast, and hops.	Pre-/Posttest
2. Describe the different stages in the brewing process.	Pre-/Posttest, Final Exam, Self-Assessment
3. Describe how beer gets its color and how this color can be measured.	Pre-/Posttest
4. Calculate the alcohol content of a beer based on initial and final specific gravity.	Pre-/Posttest, Final Exam
5. Predict how a beer's characteristics might change if its ingredients or brewing conditions are altered.	Worksheet, Final Exam, Self-Assessment
6. Apply the scientific method to the brewing process.	Pre-/Posttest, Worksheet

more likely to view beer and brewing in scientific terms. Experimental modules such as this one provide instructors with increased opportunities to highlight biology's relevance to the real world and foster problem-solving skills.

Intended audience / Prerequisite student knowledge

This lab module was designed for and implemented in an upper-division microbiology lab course, Biological Sciences MII8L, at the University of California, Irvine. This course consists mostly of fourth-year students with over 90% of the class being 21 years or older. All levels of students, including lower-division biology majors and non-majors, can easily accomplish the majority of the module, including the brewing procedure and characterization of the final beer. The most challenging aspects are the hypothesis construction and the requisite literature search necessary to develop an evidence-based prediction. While we believe this portion of the module is key to truly understanding the scientific basis of the brewing process, it may be difficult for students unaccustomed to searching for and reading scientific articles, and thus can be eliminated if deemed necessary. No specific content knowledge is required prior to the module, although a basic understanding of starch hydrolysis, fermentation, and the role of oxygen in energy generation is recommended. In terms of lab skills, it would be beneficial if students were accustomed to following proper sterile technique.

Learning time

The brewing module requires three weeks of the course. Introduction to the background information regarding the brewing process occurs at the beginning of week I and can be adequately covered in one hour. In the week I lab, students work in pairs to prepare and bottle the wort with the brewing yeast. Roughly two hours are spent completing this procedure. Students work in conjunction with another student pair to select a single beer ingredient to alter (described in detail below) and to develop a hypothesis predicting how this modification will affect the beer. In week 2, students pour

the beer into a new container to remove insoluble products and add sugar to initiate the carbonation process. Finally, in week 3, students characterize the resulting beer and fill out a worksheet, which includes predictions about the two beers produced as well as analysis of the final products. This can take up to one hour of lab time.

PROCEDURE

Materials

 All brewing ingredients were purchased from Monster Brew (www.monsterbrew.com). The specific ingredients used in our implementation of the module include:

YEAST

- Muntons Premium Gold (http://www.monster brew.com/Prod_MuntonsPremiumGoldYeast.cfm)
- Nottingham Brewing Yeast (http://www.monster brew.com/Prod_Nottingham.cfm)
- Safbrew WB-06 (http://www.monsterbrew.com/ Prod_SafbrewWB-06.cfm)

HOPS

- Centennial Hop Pellets (http://www.monsterbrew. com/Prod_Centennial.cfm)
- Chinook Hop Pellets (http://www.monsterbrew. com/Prod_ChinookhopPelletsIoz.cfm)
- Fuggle Hop Pellets (http://www.monsterbrew.com/ Prod_FuggleUSHopPelletsIoz.cfm)

EXTRACT

- Dry Malt Extract Extra Light (http://www.monsterbrew.com/Prod_DryMaltExtract-Extra Light3LB.cfm)
- Dry Malt Extract Wheat (http://www.monster brew.com/Prod_DryMaltExtract-Wheat3LB.cfm)
- Dry Malt Extract Amber (http://www.monster brew.com/Prod_DryMaltExtract-Amber3LB.cfm)
- Dry Malt Extract Extra Dark (http://www.monster brew.com/Prod DryMaltExtract-ExtraDark3LB.cfm)

- Necessary equipment includes sterile 200-mL glass bottles, 600-mL beakers, tripods, Bunsen burners, scales, sterile stirring rods, ice buckets, hydrometers, hydrometer test jars, pH paper, spectrophotometer and cuvettes.
- 3. A detailed list of reagents, including the per-student numbers and faculty instructions, are included in the supplemental materials (Appendix I).

Student instructions

Students are provided with the background information and protocol in the lab manual as well as lecture slides (Appendices 2 and 3). They work in pairs, each pair collaborating with another to design an experiment by altering one ingredient in the initial brewing process. A brief description of the protocol is as follows: Students weigh out the appropriate amount of malt extract and boil it in 250 mL of water for 45 minutes. At this point, they weigh out the hops, add it to the mixture, and continue boiling for 15 minutes. The volume is then increased to 500 mL with the addition of sterile water. The resulting mixture, the wort, is rapidly cooled in an ice water bath. A fraction of the wort is added to a container, and a hydrometer is used to determine its specific gravity. The starting pH is measured with pH paper. The remainder of the wort is added to a sterile bottle and shaken vigorously to aerate. Brewing yeast is then added to the solution, and the bottle is incubated at room temperature for a week. The following week, the beer is transferred into a new bottle to remove a majority of the insoluble products, and 5 mL of a sterile 30% sucrose solution is added. The bottle is capped tightly to ensure carbonation and left at room temperature for a week. Finally, students analyze the beer taste (using a sip-and-spit method), alcohol and calorie content, final pH, and standard reference method (SRM, color). These measurements require a hydrometer, pH paper, and a spectrophotometer.

Students complete one post-module assignment in the form of a worksheet (Appendix 4). One part of the worksheet is to predict the specific expected change in the beer based on the experimental variable. The prediction is expected to be very detailed (i.e., not merely stating that one type of extract will lead to darker beer, but instead describing the specific reasons for this difference in color) and must be supported using primary literature. The remainder of the worksheet collects the above data, requiring the students to highlight the differences between the two beers and to conclude whether their hypothesis was supported or refuted by their data.

Faculty instructions

In lecture of week I of the module, the instructor spends roughly an hour describing the brewing process and the role of the various ingredients in producing the beer. The students are also introduced to the specifics of the module, the basics of the experiment they will design along with the prediction they are expected to make, and a walk-through of the different types of data that will be collected. The slides used from this lecture are included in the supplemental materials (Appendix 3).

The lab protocols are fairly straightforward, assuming students are familiar with sterile technique, and our students were able to complete the module without major issues. It was necessary to remind the students that the boiling process will result in evaporation of water, and that additional water may need to be added to prevent caramelization of the malt. Another suggestion is to sterilize the hydrometers prior to the class tasting period, so that the beer added to the hydrometer can be returned to the original container. Because the hydrometer requires much of the produced beer, this ensures that there is a sufficient amount for the students to taste. Brewing in larger quantities would also eliminate this problem, although that would require additional reagents.

For a class of 100 students (50 pairs making beer), 150 sterile brewing bottles are needed. For our class, students were responsible for weighing out the extract and hops, although we weighed out the yeast and aliquoted the carbonation sugar into sterile vials so that students would not need to pipette and potentially contaminate a stock solution. Beer tasting may involve approval from various campus organizations (see details below).

Suggestions for determining student learning

Assessment of the student learning objectives can be completed with a variety of methods, including identical pre-/posttests (Appendix 5), self-assessment questions (Appendix 6), summative final exam questions (Appendix 6), and the worksheet to be completed at the end of the module (Appendix 4). The pre-/posttest consists of 14 content and data analysis questions that cover a number of the module learning objectives. It is important that these are administered to students under the same motivation (for example, not presenting the pretest questions for participation credit and adding the post-test questions to an exam, which may skew the data) and that the results of the pretest are not explicitly discussed to prepare students for the post-test. For the self-assessment, we chose to present the students with the statements only after module completion, as opposed to in a pre/post format. Thus, after completing the module, students noted their thoughts about their current abilities and gauged what they believed they were capable of prior to the start of the module. This was done to account for response-shift bias, which describes the fact that students may inaccurately self-assess their abilities prior to an activity due to a lack of context (10). The self-assessment can also be done prior to starting the module to generate a third data point. Course exams and the worksheet (Appendix 4) can also be used to measure student learning, although this is more difficult to do in a pre/post manner.

Sample data

Students collected a wide variety of data regarding their beer, including taste, alcohol content, calorie content, pH, and SRM (color). The protocols for how to collect these data are included in the student handout (Appendix 2). All students conducting the experiment in our course were successful in collecting all data, although the quality of beer (in terms of taste) varied from group to group according to student, teaching assistant, and instructor opinions. Alcohol content ranged from 3.6% to 7.8%, calorie content from 100.0 to 199.5 calories per 12 ounces, starting pH from 5.0 to 5.5, final pH from 4.0 to 4.4, and SRM from 4.2 to 30.2. Examples of different beers brewed by students using various malt extracts are shown in Figure 1.

Safety issues

All ingredients used for brewing have no known safety issues, and the brewing and analysis process generally do not present safety concerns. One potential issue is the tasting of the beer. Prior to incorporation of the module into the lab curriculum, we contacted the UC Irvine Associate Dean of the School of Biological Sciences, Campus Risk Manager, Environmental Health and Safety officer, and campus police department to ask for approval. All groups supported this module as long as we followed these guidelines:

- Students were not allowed to swallow the beer.
 They must only sip the beer and then spit it out.
- Only students 21 years and older, with government issued identification in hand on the day of the tasting, could participate in the tasting. These students were given a pen mark on their hand or a ribbon was tied around their wrist to identify them.
- 3. The beer was restricted to the laboratory room and students could not remove it from the room.

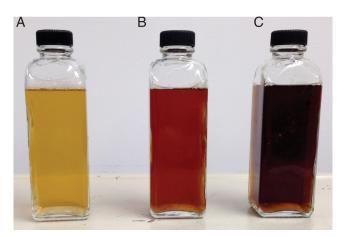


FIGURE 1. Sample brews from student groups. These beers were made using (A) extra light, (B) amber, and (C) extra dark malt extract following the protocol outlined in the methods.

4. Students had to sign a waiver with the above information along with a statement that they would act responsibly.

We made it clear to the students that tasting was not required, and that any who did not want to participate did not have to. In addition, the module should not be performed in a traditional laboratory space, to prevent accidental ingestion of pathogenic microbes. This module should be performed in a space safe for food handling.

Assessment of the module and dissemination of the data were performed in accordance with UC Irvine Institutional Review Board (IRB) approval (application #2012-9025).

DISCUSSION

Field testing

The UC Irvine Microbiology lab course schedule consists of a two-hour lecture attended by all students in the course (100–160 per quarter) followed by four hours of lab split over two days (three hours one day, one hour two days later). The course instructor leads the lecture, designs the curriculum, supervises the graduate student teaching assistants and develops the course assessments. The lab sections each consist of 20 students led by a TA, with five to eight sections per quarter.

This lab module was implemented in a course with 100 students in five lab sections. Other lab activities conducted during the quarter included isolation and identification of bacteria, antibiotic resistance, regulation of worm capture by a nematophagous fungus (16), and bacterial evolution. The beer module was scheduled for the last third of the course, and was performed simultaneously with a number of the above-mentioned experiments. By the end of the module, all pairs of students had successfully made beer. The different forms of assessment utilized throughout the module centered on whether students achieved the learning objectives. The results are discussed in the following section.

Evidence of student learning

The module learning objectives and the means of assessment for each can be found in Table I. Assessment measures included a pre-/posttest, student self-assessment, final exam and module worksheet. The pre-/posttest included questions focused on basic content knowledge and the ability to analyze data and perform relevant calculations (Appendix 5). The tests were administered in the lab sections a week before the module began (pre) and the same week as module completion (post). Students were allowed 15 minutes for each test, were awarded 0.5 points extra credit for completion, and were encouraged to answer the questions to the best of their abilities for class assessment purposes. The questions or answers were not discussed

after students took the pretest. As evident from Figure 2A, student performance for each question was significantly higher following completion of the module (p < 0.0001 for all questions).

Students were presented with a variety of statements meant to gauge self-assessment of knowledge and abilities related to brewing. Reponses were collected on a five-point Likert scale (5 = strongly agree to 1 = strongly disagree) using the iClicker personal response system during the final lecture of the course (which coincided with the completion of the module). Students were asked to rate their agreement with several statements based on their current attitudes as well as the response the student felt they would have provided at the start of the module. As previously mentioned, this was to control for response-shift bias due to a lack of context regarding these statements. We felt this was especially relevant for an upper-division biology course, as past experience has shown that our advanced students in this lab tend to feel very confident about their abilities before being immersed in a new activity (16), which may not match their actual abilities. Students' attitudes and confidence significantly increased following completion of the module (Figure 2B). An additional statement regarding interest in brewing also produced similar gains, illustrating that not only are the students more knowledgeable, they are more inclined to apply what they learned in the classroom in the real world.

A third means of assessment was the course's final exam. As the module occurred toward the end of the quarter, students were tested on the brewing process only on the final, with three questions (Appendix 6), consisting of 12% of the exam. Performance on these questions is indicated on Figure 2C. To provide a frame of reference for these scores, we compared them both with overall performance on the final exam, as well as performance on questions of similar Bloom's levels (levels 2 and 3). Bloom's taxonomy is a means to characterize different types of thinking that can be demonstrated, and while questions of similar Bloom's level are not necessarily equivalent in terms of difficulty, it is a common method currently used to compare exam questions (4). Students performed better (QI and Q3, p < 0.000I) or similarly (Q2) on the brewing related questions compared with other questions of similar Bloom's level or on the final exam as a whole. In addition, we examined the class's ability to write a hypothesis on the beer worksheet based on the single ingredient they altered. To receive credit for the hypothesis, the statement was required to be specific, supported by primary literature, and properly presented as a prediction. Based on this rubric, 83% of the class earned full credit for their work. Examples of student hypotheses can be found in Appendix 7. While both these data and performance on the final exam do not indicate that student accomplishments were specifically due to the module, they do demonstrate that students were able to fulfill the relevant learning objectives after completing the module.

Possible modifications

As previously stated, this module can be adapted for all types of students with different backgrounds. Advanced students like those in our lab were expected to provide a

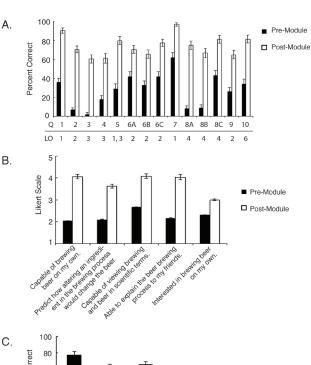


FIGURE 2. Students achieved the module learning objectives through a variety of assessments. (A) A 14-question pre-/posttest was administered before and after the beer module in laboratory sections (n = 93 students). Each question (Q) was related to the indicated learning objective (LO; shown below each question). Post-test gains are statistically significant (p < 0.0001) for each question by t-test. (B) Students noted their agreement with the following statements after completion of the module on a fivepoint Likert scale (5 = strongly agree, I = strongly disagree). At that time, they were also asked to note their agreement prior to the start of the module. Post-test gains are statistically significant (p <0.0001) by chi-square test. Questions were asked using the iClicker system during lecture (n = 86 students). (C) Student performance on three module-related questions on the final course exam are indicated (QI-3, n = 95 students). For comparison, performance on the same exam for other questions of similar Bloom's levels (levels 2 (comprehension) and 3 (application)), as well as overall performance on the final exam, are indicated. Performance on QI and Q3 is significantly higher compared with the other Bloom's level 2 and 3 questions and compared with the overall exam performance (p < 0.0001) by t-test. Error bars indicate the standard error of the mean for all figures.

detailed prediction of how a specific component would alter the beer characteristics and the molecular basis for this prediction, based on their findings in the scientific literature. These expectations can be relaxed for students less familiar with searching for primary literature to include other sources of information, as there is considerable brewing material from non-peer-reviewed sources on the Internet.

The final assessment can also be more rigorous than the worksheet used for our purposes to instead include a lab report formatted like a scientific article. The choice of final assessment will depend on the course workload at the time of module's implementation. As far as scheduling, the length of time brewing and carbonating the beer can also be increased. We have brewed using two-week time periods for each without negatively impacting the beer produced.

An additional characterization component is the determination of yeast population size after the fermentation process has been completed, as the number of yeast in the beer has an impact on fermentation. This can be performed either by serial dilution or with a spectrophotometer. Serial dilution can be performed as follows: (I) Remove I mL of the resuspended beer (so that the yeast are not settled at the bottom of the bottle). (2) Create a 1:10 dilution of the yeast/wort solution in sterile water. (3) Continue making 1:10 serial dilutions until 8 in total have been created. (4) Plate 10 μL of each dilution onto a yeast peptone dextrose (YPD) plate. (5) Incubate plate at room temperature or 30°C for I to 2 days. Identify which plated dilution allows for counting of single colonies to calculate the colony forming units (CFU) per mL. The spectrophotometer method can be performed as follows: (1) Remove 1 mL of the resuspended beer. (2) Create a 1:10 dilution of the yeast/wort solution in wort lacking yeast (the plain wort solution will also be used to blank the spectrophotometer). (3) Determine the optical density of the dilution at 600 nm. (4) Determine the number of cells per mL in the beer using the following conversion factor: I mL of a 1.0 OD_{600} solution contains roughly 1 × 10^7 cells per mL.

For future implementations of the module, we plan to incorporate more creativity components. We will ask students to generate names and bottle labels for their brews and add an instructor-judged tasting contest between the different groups with a prize for the winner. These activities will hopefully further connect microbiology and specifically the science behind brewing with practical applications seen in society.

SUPPLEMENTAL MATERIALS

Appendix I: Reagent/equipment list and faculty instructions

Appendix 2: Student handout and protocol

Appendix 3: Lecture slides
Appendix 4: Beer worksheet
Appendix 5: Module pre/post-test

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Appendix 6: Self-assessment and final exam questions

Appendix 7: Examples of student hypotheses

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