A Biology Laboratory Exercise Using Macromolecule Assays to Distinguish Four Types of Milk

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INTRODUCTION

One of the drawbacks of cookbook-style laboratory exercises for General Biology courses is that students are not challenged to develop skills in scientific reasoning, such as formulating hypotheses and designing and carrying out experiments. Several traditional laboratory curricula (2, 3, 4) include exercises involving semi-quantitative colorimetric assays to detect proteins (biuret test), reducing sugars (Benedict’s test), starch (Lugol’s test), and lipids (Sudan red test) in a variety of easily prepared solutions (glucose, albumin, glycine, etc.) and familiar food items (lemon juice, cornstarch, egg white, etc.). These lab exercises typically incorporate the identification of an unknown substance based on its behavior in the various macromolecule assays.

An extension of this lab exercise was developed to allow students to use their knowledge of the macromolecule assays to design an experiment to distinguish four types of “milk”: whole milk, skim milk, cream, and soy milk (rice milk or almond milk could also be included). Students must build on their existing knowledge (e.g., how skim milk is prepared), understand the usefulness of the four assays (e.g., the biuret test identifies substances containing protein), choose which of the four assays to perform, and select appropriate negative and positive control substances based on the results of the exercises already completed.

PROCEDURE

The protocol is designed to minimize the need for specialized lab equipment. Each group of four students requires about 20 5-mL test tubes in a rack, a hot plate and 400-mL beaker for preparing a boiling water bath, 9-cm-diameter filter paper, a large glass petri dish, and plastic dropper pipets. A vortex mixer and handheld hairdryer are helpful.

In the first half of the lab period, students carry out four macromolecule assays. Students add 20 drops (~ 1 mL) of biuret reagent (0.25 mM CuSO₄ in 10 M NaOH) to 20 drops of each test substance and use the appearance of a purple color to confirm the presence of protein. To detect reducing sugars, students add 20 drops of Benedict’s reagent (1) to 20 drops of each test substance, boil the tubes for 10 min, and note the appearance of an orange, red, or brown precipitate. To assay for starch, students add 5 drops of Lugol’s solution (0.3% I₂, 0.7% KI) to 20 drops of each test substance and look for a dark blue color. For the Sudan red assay, students blot a small drop of each test substance onto filter paper, allow the drops to dry (a hairdryer helps), soak the paper in a petri dish containing 0.2% Sudan IV for 5 min, rinse and dry the paper, and use the presence of a dark red spot to confirm the presence of lipid. Bottles are provided to collect waste material that contains copper from the biuret reagent and Benedict’s reagent. Note that Sudan red is a possible carcinogen and should be handled with gloves.

In the second half of the lab period, students are provided four “milk” samples identified only by code letters. Each group discusses what types of macromolecules are likely to be present in whole milk, skim milk, cream, and soy milk. Students then decide which of the four assays to perform to differentiate these substances, and they formulate a hypothesis that summarizes their predictions about how each substance will behave in the assays. After carrying out the assays, which must each include a negative control (distilled water) and a positive control (a substance that gave a strong positive result during the first part of the lab exercise), students interpret their results and identify the four milk samples.

A successful experiment consists of two assays: the Sudan red test plus either Benedict’s test or Lugol’s test. The Sudan red assay reveals that cream contains the highest concentration of lipid; whole milk contains somewhat less, and skim milk and soy milk contain the least (Fig. 1). Soy milk can then be distinguished from skim milk (and whole milk and cream) by Benedict’s test (Fig. 2), since the dairy products contain the reducing sugar lactose while soy milk typically contains undetectable levels of reducing sugars. Alternatively, Lugol’s test reveals the presence of starch in soy milk and its absence in the dairy products (Fig. 3). The biuret assay is not definitive, since all samples except cream contain detectable protein, and the assay cannot distinguish between whole milk and skim milk.

CONCLUSION

Macromolecule assays are a useful starting point for students to design additional experiments, because such assays are easy to carry out and generate reliable results.
In performing the macromolecule assays to distinguish four types of milk, students must apply the scientific method. Students also learn that they must build on existing knowledge, that there may be more than one way to obtain an answer, and that they must work as a team to make predictions and plan the experiment. The entire exercise can be completed in 40 to 60 minutes.

A review of students’ lab reports reveals that virtually all groups choose to perform the Sudan red test for lipids. Approximately two-thirds perform the Sudan red test plus either Lugol’s test or Benedict’s test. The remainder perform just one test, which is inadequate, or three tests, which is more than necessary. The student success rate suggests that the exercise is sufficiently challenging.

An instructor could award points for the simplest possible procedure (for example, Benedict’s test and Lugol’s test both differentiate soy milk from the dairy products, but Lugol’s test is easier and faster) and deduct points if students perform more than two assays or fail to include the appropriate controls.

Students can often guess which unknown is cream (based on its consistency, even when diluted 1:1 with water) and which is soy milk (which may be a faint tan color). However, by the time students actually see and handle these substances, they have already formulated their hypotheses and made an experimental plan, so their results confirm their suspicions.

REFERENCES