Sucrose & Glucose & Fructose, Oh My! Uncovering Hidden Sugar in Your Food

https://www.sciencebuddies.org/science-fair-projects/project-ideas/HumBio_p035/human-biology-health/sugar-metabolism (http://www.sciencebuddies.org/science-fair-projects/project-ideas/HumBio_p035/human-biology-health/sugar-metabolism)

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Experimental Procedure

Testing the Glucose Strips

In this part of the science project, you will create **controls**, or samples with known ingredients that should give clear, expected results. You will do this to make sure that the glucose test strips are working properly. If the test strips are not working properly, then the rest of this experiment will not work. The **positive controls** will contain different concentrations of glucose. The **negative control** will be a sample without glucose.

1. First, make the positive controls using water and the glucose powder. To do this, make a **dilution series** using sequential twofold dilutions to create the following concentrations: 2%, 1%, 0.5%, 0.25%, 0.125%, and 0.0625%.
    a. Label six cups: 2%, 1%, 0.5%, 0.25%, 0.125%, and 0.0625%.
    b. Add 4 grams (g) of glucose to 200 mL of water in the cup labeled 2% and stir until the glucose dissolves.
    c. Optional: Add 2–5 drops of food coloring to the 2% glucose solution. The color does not matter. *Note*: The food coloring will allow you to keep track of your dilution levels as the color of each dilution will get less intense. It does not interfere with the glucose measurements.
    d. Add 100 mL of water to the other five cups.
    e. Measure 100 mL of the 2% solution and add it to the cup labeled 1% to make a 1% solution. Stir well.
    f. Measure 100 mL of the 1% solution and add it to the cup labeled 0.5% to make a 0.5% solution. Stir well.
        i. Between each dilution, make sure to rinse and shake the excess water from the graduated cylinder or container you are using to transfer the 100 mL volumes. Also, use a clean stirrer.
    g. Repeat this process for the remaining dilutions.
        i. When you are done, each cup should have 100 mL of liquid, except for the 0.0625% solution, which should have 200 mL.
2. Fill an extra cup with 100 mL water. Do not add any of the glucose solutions to it. Label it 0%. This will be your negative control.
3. If you used food coloring for your dilution series, you should now have seven cups that look similar to the ones in Figure 2.

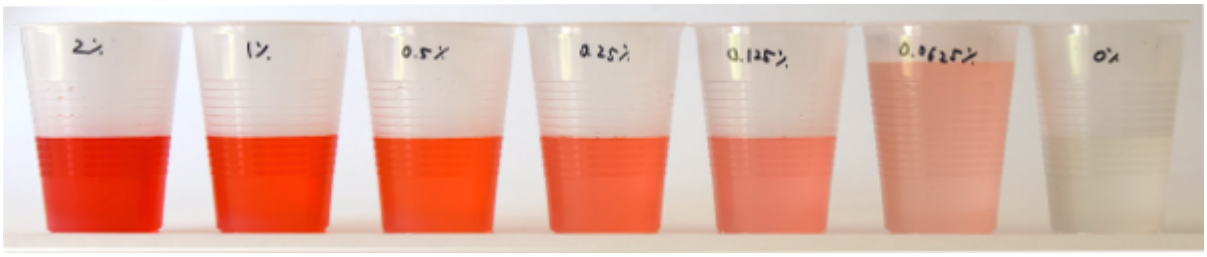

Figure 2. If you used food coloring (in this picture, red food coloring was used), the glucose dilution series should look like the ones in this picture (arranged by most concentrated to least, from left to right). (Each cup should have 100 mL of liquid, except for the 0.0625% solution, which should have 200 mL.) A seventh cup, serving as the negative control, should only contain water (on the far right in this picture).

4. Dip a test strip into each of the seven cups, one at a time. After 1–2 seconds, remove the test strips from each solution and watch them for 30 seconds (which should be the time recommended in the test strip instructions). Then match the color of the glucose marker on the test strip to the color on the bottle shown in Figure 3. Do the colors match what you would expect? Write down your observations in your lab notebook. *Note*: For high glucose concentrations, it might take up to 60 seconds until the color matches the actual concentration. Therefore, we recommended you dilute your samples once they approach a glucose concentration of 1%.

a. See the Technical Note for guidance on matching the color of the glucose test strips to the color on the bottle.
b. If the color changes to the maximum range (2%) before 30 seconds, list it as greater than 2% (">2%"). You do not need to perform a dilution.
c. If you do not have a clear color change for any of the positive control solutions with a concentration greater than 0.0625% repeat the procedure. If the second time it is still problematic, you might have to buy new test strips. It is ok to have a slightly lower reading for the pure glucose solutions. Remember, these test strips were designed for measuring low concentrations of glucose in a urine sample so the results might be slightly different for pure glucose solutions. If the test strips for the glucose solutions at 30 seconds are more than one color off from what it is expected to be (for example, if the 1% solution reads less than 0.5% or the 0.25% solution reads greater than 0.5%), you could adjust the readout time accordingly (for example to 60 seconds). However, you have to make sure that throughout the experiment, you keep the same readout time for all of your samples.
d. *Tip:* If you would like additional help with reading the glucose test strips, check out the Frequently Asked Questions (FAQ) (#help) for this science project.

Technical Note

When matching the color of a glucose test strip to a color on the bottle, keep in mind these helpful tips:

- The colors on the bottle will not exactly correspond to the percent glucose solutions you made. There will probably be colors for 0% ("Negative"), 0.1%, 0.25%, 0.5%, 1% and 2% glucose solutions, as shown in Figure 3.
- Some test strip colors may fall between two of the colors on the bottle, for example between 0.5% and 1%. If this happens, write down the two numbers in your lab notebook and calculate their average.
- If the color changes to the maximum range (2%) before 30 seconds, list it as greater than 2% (">2%"). Depending on where this happens in the Experimental Procedure, you may need to then perform a 1:10 dilution and re-test the sample. You will get more accurate results if you start diluting your samples once the glucose concentration is getting close to 1%. There are two ways in which you may perform a 1:10 dilution, and the preferred way will be specified in the text:
  - Use a transfer pipette to add nine drops of water and one drop of the test solution on a bottle cap. Rinse the transfer pipette in between each sample.
  - Mix 1/2 teaspoon (tsp.) (2.5 mL) of the sample with 22.5 mL water to make a 1:10 dilution. (Note: You will only test 15 mL of this dilution.)

Remember that if the 1:10 dilution reading reports 1% glucose, then the glucose in the sample is really 10%, because it was diluted tenfold.

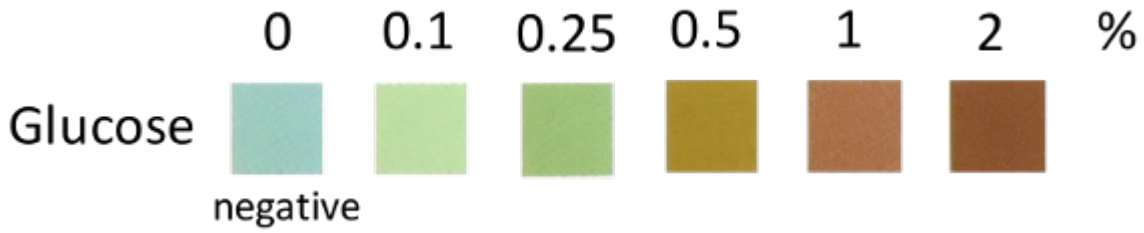

Figure 3. This is the color chart for glucose on the test strip bottle. After a glucose test strip is dipped in a glucose solution and removed, its color should change and match a color on its bottle (or be between two colors). The color on the bottle will indicate the percentage of glucose in the solution tested.

Testing Invertase Activity

In this next part of the science project, you will test the activity of the invertase enzyme. It is important for you to do this step so that you know how long you should test your selected foods with the invertase enzyme. You will test the activity of the invertase enzyme by investigating how long it takes it to turn a known amount of sucrose (in solution) into glucose. You can look at Figure 1 in the Introduction (#background) to see the chemical reaction. When invertase is added to the sucrose solution, the concentration of glucose should increase over time as the sucrose is converted to glucose. However, after some amount of time, the concentration of glucose will appear to remain the same, or plateau. For an example, see Figure 4. Although the invertase may still be converting sucrose to glucose, it is doing it at an extremely reduced rate. This is probably partly due to **product inhibition**, which is when the product of a reaction (glucose in this case) stops the enzyme (invertase) from making more product. Here you will determine how much time is needed for the invertase enzyme to convert some, but not all, of the

sucrose in a 10% solution, before product inhibition occurs and the reaction clearly slows down. This part of the project is an example of the study of enzyme kinetics.

1. Make a solution containing 10% sucrose.
    a. Fill a cup with 60 mL of water.
    b. Add 6 g of sucrose to the cup of water. Mix until all the sucrose dissolves.
    c. Put 15 mL (1 Tbsp) of this solution into a new cup.
        i. How many grams of sucrose are in 15 mL of the 10% solution?
2. In your lab notebook, make a table for recording your data. You will be taking glucose readings over time to see how much sucrose has been converted to glucose by the invertase enzyme.
    a. Starting at zero, plan on taking glucose readings every 5 minutes for the first 30 minutes, and then every 10 minutes after that. Plan on taking readings for 90 minutes total.
3. Use a glucose test strip to determine the concentration of glucose in the sucrose solution, as you did in the "Testing the Glucose Strips" section in step 4. Write your result in the table in your lab notebook under "0 minutes."
    a. There should be 0% glucose in the sucrose solution.
4. Set a timer for 90 minutes or make sure a clock is nearby.
5. Get one of the bottles from the sugar metabolism kit that contains 1 g of powdered invertase and prepare it for the experiment. *Note*: You can prepare the invertase solution right before you start the experiment, however, the invertase works better if you prepare the solution one day in advance. You do not have to prepare both of the invertase bottles at the same time. You can keep the second bottle for future experiments or prepare it once you run out of invertase in this experiment.
    a. Fill the measuring cylinder with 25 mL of distilled water.
    b. Open the invertase bottle and add the water to the invertase powder.
    c. Then close the lid and shake the bottle until all the powder has dissolved.
    d. **Important**: Once rehydrated, the invertase solution needs to be kept in the refrigerator when not used.
6. Add 15 drops (about 0.75 mL) of invertase to the sucrose solution. Quickly mix the solution.
7. Start the timer or write down the exact time in your lab notebook.
8. Use the glucose test strips to take glucose readings of the solution over time, as described in step 2.
    a. Write your results in the table in your lab notebook.
    b. See the Technical Note for guidance on matching the color of the glucose test strips to the color on the test strip bottle.
    c. Remember that the glucose readings are most accurate if you dilute your sample once the glucose concentrations reach about 1%. If the color changes to the maximum range (2%) before 30 seconds, list it as greater than 2% (">2%") and quickly perform a 1:10 dilution using 1 drop of your test solution (as described in the Technical Note on the third bullet point) to determine the actual percentage of glucose in the sample. Take a glucose reading of the 1:10 dilution.
    d. When the glucose reading has remained the same for at least 20 minutes (3 readings spaced 10 minutes apart), you can stop taking readings.
9. Graph your results. Put the time on the x-axis and the glucose concentration on the y-axis.
    a. You should end up with a graph that roughly looks similar to Figure 4.

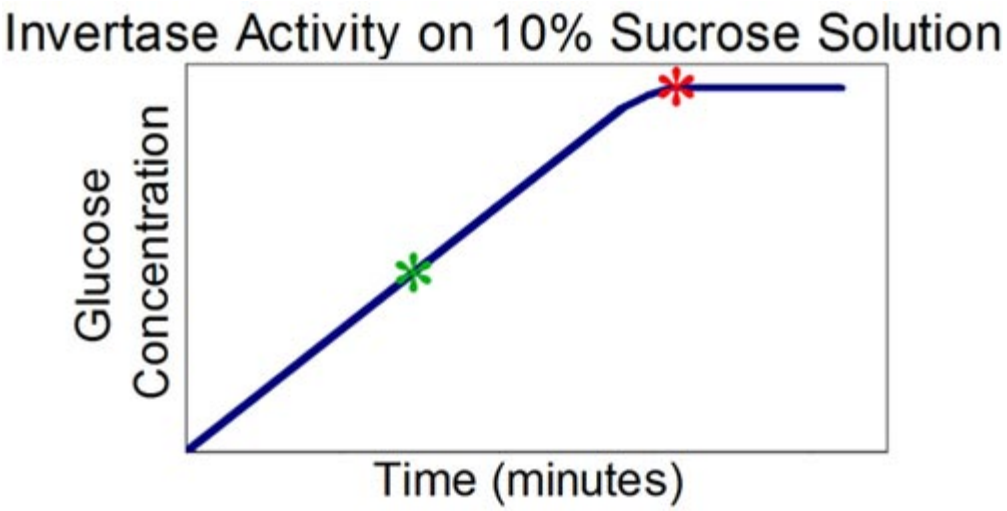

Figure 4. Initially, the invertase enzyme converts sucrose to glucose at a roughly constant rate, creating a linear, or nearly straight, line. However, after some amount of time, the concentration of glucose appears to remain roughly the same, or plateau. Although the invertase may still be converting sucrose to glucose, it is doing it at an extremely reduced rate. The red asterisk marks where the glucose concentration first leveled off. The green asterisk, which we will call the linear time point, marks the time point at which the glucose concentration was half what it was at the red asterisk. Your actual data may only roughly match the line in this graph.

10. Analyze your results.
    a. When the glucose concentration first leveled off, what was the glucose reading?
        i. For example, in Figure 4, this point is marked with a red asterisk.
    b. Divide this concentration in half. At about what time was this glucose reading taken? Write this time in your lab notebook as the "**linear** time point."
        i. For example, in Figure 4, this point is marked with a green asterisk.
11. The "linear time point" you determined in step 10.b. is the time you will use to test your selected foods.
    a. This is because this time point is when the invertase enzyme is clearly making the reaction occur at a roughly constant rate (it is a point on a linear, or nearly straight, line). Also, it is well before the reaction starts slowing down, which can cause inaccuracies in measuring the glucose concentration.

Testing the Foods for Glucose Concentration Before and After Adding Invertase

Now that you have determined your linear time point, you are ready to test your selected foods. In this part of the science project, you will first test the glucose concentration of your selected foods and then react each with invertase to determine how this changes their glucose concentrations.

1. In your lab notebook, make a table for recording your data.
    a. For each food sample, you will take two glucose readings: *one before* adding the invertase and *one after*, at the linear time point you determined in the "Testing Invertase Activity" section in step 10.b.
    b. Test at least three different samples of each selected food.
        i. Multiple trials help scientists make sure that their results are accurate and reproducible.
2. Label the cups with the food samples you will test.
3. Let all of the food samples you will test come to room temperature before testing them.
    a. The activity of an enzyme is affected by temperature. It is important that all of the test foods are about the same temperature, so that any differences you see in your data are not because the foods were of differing temperatures.
4. For foods with high amounts of sugar, such as soft drinks (not diet), or viscous substances, such as syrup, molasses, baby food, or peanut butter, dilute the samples 1:10 in water before testing them. Make the 1:10 dilution using 1/2 tsp. of your sample (as described in the third bullet point of the Technical Note).
5. To each cup, add 1 Tbsp. (15 mL) of the food that you will test.
6. Use a glucose test strip to determine the concentration of glucose in each food sample, as you did in the "Testing the Glucose Strips" section in step 4.
    a. See the Technical Note for guidance on matching the color of the glucose test strips to the color on the bottle.
    b. If the color changes to the maximum range (2%) before 30 seconds, list it as greater than 2% (">2%") and perform a 1:10 dilution using 1/2 tsp. of your sample (as described in the third bullet point of the Technical Note). Use the diluted sample for all tests.
7. Write the glucose concentration for each sample in your table.

8. Set a timer for the linear time point you determined or make sure a clock is nearby.
9. Add 15 drops (about 0.75 mL) of invertase to each food sample. Quickly mix the samples.
    a. Start only a few samples at a time so it is easier to manage them.
    b. Other than taking it out to quickly add to the samples, the invertase solution should remain in the refrigerator.
10. Start the timer or write down the exact time in your lab notebook.
11. At the linear time point, use a glucose test strip to determine the concentration of glucose in each sample. Write this in your table.
    a. See the Technical Note for guidance on matching the color of the glucose test strips to the color on the bottle.
    b. If the color changes to the maximum range (2%) before 30 seconds, list it as greater than 2% (">2%") and perform a 1:10 dilution using one drop of your test solution (as described in the third bullet point of the Technical Note). Take a glucose reading of the 1:10 dilution.
12. Graph your results. Make a bar graph and put the food names of the samples on the x-axis and the glucose concentration on the y-axis. Include both glucose readings for each sample (before adding invertase and at the linear time point).

Analyzing Your Results

1. Look at the graph you made in the "Testing the Foods for Glucose Concentration Before and After Adding Invertase" section (step 12). Do the glucose readings you took before adding the invertase match what you would expect for these foods?
    a. Which foods had the most glucose before adding the invertase? Which had the least? Did any have no glucose?
2. Using the data you collected, you can determine the sucrose concentration in each of the foods you tested.
    a. Look at the graph you made in the "Testing Invertase Activity" section. At the linear time point, what was the glucose concentration? Using this result, you can find out how much of the original sucrose has been converted at that point. We will call this value "Percentage of sucrose converted." To calculate this, you have to divide the glucose concentration measured at the linear time point by the original sucrose concentration. As in this project the concentration is always expressed in percent (%), you have to also multiply by 100. You can see this equation written out in Equation 1.
        i. Here is a sample calculation: if at the linear time point the 10% sucrose solution had a reading of 2% glucose, then this means that 20% of the total sucrose (2 divided by 10, which is 0.2, multiplied by 100 to obtain the percentage) had been converted to glucose.

    Equation 1:

$$Percentage\ of\ sucrose\ converted = \frac{Glucose\ concentration\ at\ linear\ time\ point}{Original\ sucrose\ concentration} \times 100$$

    b. Look at the data you collected in the "Testing the Foods for Glucose Concentration Before and After Adding Invertase" section. To determine the original sucrose concentration of each of your food samples, use Equation 2, which is the same as Equation 1 but solved for the original sucrose concentration. This time you have to divide the glucose concentration at the linear time point by the percentage of sucrose converted (which you just determined). Again, you have to include 100 as a multiplier because you want your final result to be in percent.

    Equation 2:

$$Original\ sucrose\ concentration\ = \frac{Glucose\ concentration\ at\ linear\ time\ point}{Percentage\ of\ sucrose\ converted} \times 100$$

        i. Here is another sample calculation: if at the linear time point a food had a 2.5% glucose reading, and we calculated that the percentage of sucrose converted is 20%, then according to Equation 2, the original sucrose concentration of this food was 12.5% (2.5 divided by 20, which is 0.125, multiplied by 100 to get the percentage).
    c. Which food(s) had the highest original sucrose concentration? Which food(s) had the lowest? Did any foods have no sucrose? Do your results match your predictions?
3. How did the sucrose concentration in the different foods affect the glucose concentration at the linear time point?
4. How might this experiment be different from what takes place in the human digestive system? Do you think that even more glucose might have been made at the linear time point due to other chemical reactions taking place?
    a. *Hint:* Re-read the Introduction (#background).
5. How do your results compare to the amount of sugar listed for these foods on their packaging?
    a. You can also take a look at the "Ingredients" to see what types of sugars might be in the foods. High-fructose corn syrup actually contains fructose and glucose.

6. Knowing the sugar contents of different foods is particularly important for someone with diabetes. If someone has hypoglycemia and needs a fast glucose boost, which foods would you recommend eating? Which foods would you recommend avoiding? Which foods would you recommend eating in moderation, not only because they are high in glucose, but also because they are high in sucrose? Which foods may be safe for someone with diabetes to consume because they do not change blood glucose levels that much?

Frequently Asked Questions (FAQ)

FAQ for this Project Idea available online at https://www.sciencebuddies.org/science-fair-projects/project-ideas/HumBio_p035/human-biology-health/sugar-metabolism#help.