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#19 Analysis of the Sugars in Soft Drinks

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Introduction

Why analyze the sugars in soft drinks? "Sugar" is a loosely used term, which can mean many distinctly different chemicals. Labels on soft drink cans and bottles are a good example of this murky terminology—"high fructose corn syrup and/or sugar."

Prior to 1984, soft drinks in the U.S. were sweetened with cane or beet sugar, invert sugar, or corn syrups or mixtures of all of these. By late 1984, however, the majority of the beverage manufacturers had switched to "high fructose corn syrup", because it was cheaper and easier to handle. Additionally, since it was made from corn, it was not subject to the price fluctuations of the cane sugar market. (1)

This major change in the choice of sweetener came about because of the development of economical methods of enzymatically converting cornstarch to glucose and then converting the glucose into fructose. Glucose and fructose are isomers of each other, and have the same chemical formula, $C_6H_{12}O_6$. An enzyme called an "isomerase" does the glucose-to-fructose conversion job. The so-called "high fructose corn syrup" is actually a mixture of 55% fructose, 41% glucose and 4% higher carbohydrates. (Other commonly available types of syrups contain 42% fructose or 95% fructose.) (1) Fructose is actually much sweeter tasting than sucrose or glucose, and these high fructose syrups are now found in many food products, including the familiar Karo® corn syrup, which formerly was a mixture of glucose, maltose, and dextrin.

Methods of Analysis:

Analytical methods originally meant for one kind of sample can quite often be adapted for use with other kinds of samples. Of course, adaptation may require some tinkering with the reagents, concentrations, times, temperatures, etc. This idea is illustrated by the following:

1. A blood glucose analysis (Sigma Procedure No. 510) was adapted for use with soft drinks.
2. A very old quantitative "wet chemical" method (1933), originally used for determining blood glucose, can be adapted to determine the total amount of reducing sugars in various foods, including soft drinks. This method is also called the Shaffer-Somogyi method, and is a standard procedure listed in the AOAC handbook.(3,4,5)

(Note: Reducing sugars reduce alkaline copper (II) ions to copper (I) ions, while being oxidized to sugar acids such as gluconic acid. Sucrose, because of its unique molecular structure, is **not** a reducing sugar. However, all other sugars commonly found in foods—glucose, fructose, galactose, mannose, lactose and maltose—**are** reducing sugars.)

Analytical rationale:

The total amount of reducing sugars (glucose plus fructose) **minus** the amount of glucose **equals** the amount of fructose in these analyses of soft drinks.

Qualitative Tests for Glucose and Fructose:

A. Taste Tests: While some lab supervisors would strongly resist having students taste ANY chemicals, it can be a very instructive demonstration for the students to taste tiny amounts of fructose, glucose, and sucrose. The procedure is quite simple and safe and can be carried out in a regular classroom or a cafeteria.

About 1 teaspoon of each dry sugar is placed on a clean piece of plastic wrap, next to a clean cup of tap water and a pile of clean toothpicks. An empty cup marked "Used toothpicks" is also provided at each sugar station. A drinking cup containing tap water is provided for each student. The student first rinses his/her mouth with tap water. Next a clean toothpick is dipped into the water next to the sample and then into the pile of the sugar. Then the toothpick is placed on the tongue, so that a concentrated amount of sugar can be tasted. This toothpick is then discarded. Repeat the process for each sugar, rinsing the mouth in between sugar samples. Students record their impressions, and are usually quite surprised to experience the fact that in order of sweetness, fructose > sucrose > glucose.

B. Qualitative Chemical Tests: Students can do these two qualitative tests to show that soft drinks contain both glucose and fructose.

Glucose: Use Diastix test strips made by Niles Inc., Diagnostic Division, Elkhart, IN. These strips are used by diabetics to test their urine and are available from pharmacies or scientific supply companies. The strips contain a unique enzyme system which is specific for glucose. When dipped into any liquid containing glucose, the original blue-green color changes to a brownish color. The darker the brown color, the more glucose present.

Fructose: Use Seliwanoff's Test to show the presence of fructose.(6)
Seliwanoff's Reagent: 0.05 g of resorcinol in a solution of 33 mL of con. HCl in 66 mL of water.(7) Also prepare 1% solutions of fructose, glucose, and sucrose.

Seliwanoff's Test is based on the fact that fructose (a ketohexose) will be dehydrated faster with hot HCl than the corresponding aldohexoses such as glucose, mannose, or galactose. During the same time that dehydrated fructose reacts with resorcinol to produce a bright red product, the other aldohexoses give only a pale pink color. After several minutes of heating, sucrose **will** eventually give a positive test, because the HCl hydrolyzes the sucrose into fructose and glucose, and the fructose then gives the bright red color. Thus, timing is extremely critical in this test.(6)

1. Place 4 mL of Seliwanoff Reagent in each of four test tubes, labeled Blank, Fructose, Glucose, and Sucrose.
2. Add 1 mL of water to the blank and 1 mL of each test solution to the proper tubes.
3. Place tubes in boiling water bath (MUST be boiling!) for exactly 60 seconds. Observe and record any color changes. (Note: fructose = pink-red; glucose = clear; sucrose = faint pink.)
4. Continue heating and observe any color changes at 1 minute intervals for 5 minutes. (Why does sucrose eventually turn dark pink?)
5. Test 1 mL of a soft drink with Seliwanoff's Reagent and record color. Does this show the presence of fructose?

Quantitative Analysis of Glucose (adapted from Sigma Procedure 510): (8)

Summary of the Method: (quoted from the Sigma instruction brochure)
"Enzymatic methods provide a high degree of specificity in estimating blood glucose. In 1956, Keston(9) proposed the simultaneous use of glucose oxidase and peroxidase coupled with a chromogenic oxygen acceptor [e.g., *o*-dianisidine or *o*-toluidine] for the colorimetric determination of glucose in biological fluids.....The sample is added to a mixture containing glucose oxidase, peroxidase and *o*-dianisidine. The reaction is allowed to proceed to completion in approximately 30 minutes at 37° C. The final color intensity is proportional to the glucose concentration. A distinct advantage of this procedure is that precise timing is not necessary."

Principle of the reaction: (quoted from the Sigma brochure)

$$\text{Glucose} + 2\,H_2O + O_2 \xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + 2\,H_2O_2$$

$$H_2O_2 + o\text{-Dianisidine} \xrightarrow{\text{Peroxidase}} \text{Oxidized } o\text{-Dianisidine}$$
[Colorless] [Brown]

Reagents provided in the Sigma Kit:
PGO Enzymes (glucose oxidase and peroxidase) (Caution: Irritants!) in capsules
o-Dianisidine dihydrochloride, (Caution: Toxic!), vial containing 50 mg
ß-Glucose standard solution,100 mg/dL (5.56 mmol/L) in 0.1% benzoic acid solution

Solution Preparation: (from the Sigma instruction brochure)
"The ENZYME SOLUTION is prepared by adding the contents of 1 capsule of PGO Enzymes (Cat. No. 510-6) to 100 mL of distilled water in an amber bottle. Invert bottle with gentle shaking to dissolve.

COLOR REAGENT SOLUTION is prepared by reconstituting one vial of o-Dianisidine Dihydrochloride with 20 mL of distilled water.
COMBINED ENZYME-COLOR REAGENT SOLUTION is prepared by combining 100 mL of Enzyme Solution and 1.6 mL of Color Reagent Solution. Mix by inverting several times or with mild shaking.
Glucose Standard Solution is supplied ready to use."

Keep all reagents and solutions refrigerated.

Making the Standard Curve: The absorbance of the standard solutions is used to construct a "Standard Curve," from which the amount of glucose in a sample can be read.

1. Prepare solutions containing 0.5, 0.4, 0.3, 0.2 and 0.1% anhydrous glucose solutions.
2. Make 1:20 dilutions of each of the above solutions.
3. Label six tubes or cuvettes for the Blank and the five standards.
4. To the Blank, add 0.5 mL water, and to each standard tube add 0.5 mL of the 1:20 dilutions of the appropriate standards.

5. Add 5.0 mL of the Combined Enzyme-Color Reagent Solution to each of the tubes and mix thoroughly.
6. All tubes are then incubated at 37°C for 30 minutes or at room temperature [18-26°C] for 45 minutes. The incubation times can vary ± 5 minutes.
7. The absorbance of each standard is read at 475 nm on the Spec 20. (It is recommended that each lab determine the spectrum of the colored reagent, so that the maximum absorbance can be chosen. This may range from 425-480 nm.)
8. Construct a Standard (Calibration) Curve, using the Concentration of Glucose on the abscissa and the Absorbance of each of the standards on the ordinate. When a line is drawn through these points, it will be found to be linear and should pass through the origin.

Determination of Glucose in Soft Drink Samples:

The first step is to find out how much to dilute the soft drink samples. For instance, one could make several dilutions from 1:10 to 1:100. Each of these is then diluted 1:20 for the purposes of this analysis.

[Note: If one wishes to skip this first step, just make a 1:25 dilution of each soft drink, and then a 1:20 dilution of each original dilution.]

Once an appropriate dilution has been found, follow the procedure as given under "Making the Standard Curve." Use a Blank as before, and use 0.5 mL of the proper dilution of the soft drink. When the absorbance is found, read the Concentration of Glucose from the abscissa and calculate the amount of glucose/mL of soft drink, taking into consideration the dilutions used. From this, the percent or the mass of glucose in a can or bottle of soft drink can be calculated.

Note: This method can be used for any sweet drink, such as orange juice, apple juice, etc. The color of the drink will probably not interfere with the analysis, since the sample has to be diluted about 400-500 times.

Quantitative Determination of Reducing Sugars in Soft Drinks (3,4,5):

Summary of the Method: Shaffer and Somogyi introduced this method in 1932 for the determination of reducing sugars in solutions or in biological fluids. The chemistry of the method is described as follows:

1. Copper is reduced from Cu^{+2} to Cu^{+1}, using an alkaline copper solution (see below under Reagents):

$$Glucose + Cu^{+2} \xrightarrow{OH^{-1}\ (\text{boiling})} Gluconic\ acid = Cu^{+1}\ (as\ Cu_2O)$$

Note: Fructose reacts almost like glucose, and a mixture of the two can be analyzed in the same manner.

2. The solution is cooled and acidified with 0.5 M H_2SO_4, and iodine is released:

$$IO_3^{-1} + 6H^{+1} + 5I^{-1} \longrightarrow 3H_2O + 3I_2\ (brown)$$

3. The iodine reoxidizes the Cu^{+1} to Cu^{+2}:

$$2Cu^{+1} + I_2 \longrightarrow 2Cu^{2+} + 2I^{-1}$$

4. The excess iodine is titrated with 0.005N $Na_2S_2O_3$, using starch as the indicator:

$$I_2 + 2S_2O_3^{-2} \longrightarrow 2I^{-1} + S_4O_6^{-2}$$

5. A blank titration is also done in exactly the same way, with no sugar present.

6. The <u>difference</u> between the two titrations give the amount of iodine used to reoxidize the Cu^{+2}, and thus gives the amount of glucose present in the sample.

7. A calibration curve is constructed, using the percent glucose on the abscissa and the corresponding volume of thiosulfate solution on the ordinate.

   <u>Note:</u> This method is applicable not only to soft drinks, but also to honey and syrups, fruits and vegetables (after suitable grinding and extraction) and milk or milk products. In the case of milk/milk products, the reducing sugar being determined is lactose, and requires a longer heating period (30 min. vs. 15 min.), since lactose reacts more slowly. The calibration curve is made with lactose instead of glucose. The proteins in milk are removed by adding $CuSO_4$ and 0.1 N NaOH. (5)

<u>Preparation of Reagents:</u>

1. COPPER REAGENT: Dissolve the following chemicals in the order given in about 800 mL of water in a 1-liter volumetric flask:

- 7.5 g $CuSO_4 \bullet 5\ H_20$
- 25 g $KNa(C_4H_4O_6) \bullet 4\ H_20$ (Rochelle Salt)
- 25 g Na_2CO_3 (or 29.25 g $Na_2CO_3 \bullet H_2O$)
- 20 g $NaHCO_3$
- 5 g KI

  Next, accurately weigh 0.785 g of KIO_3, dissolve in 20-30 mL water, rinse quantitatively into the volumetric flask and dilute with water to 1 liter. Mix well.

2. SODIUM THIOSULFATE, O.1 N: Dissolve 25.00 g of $Na_2S_2O_3 \bullet 5\ H_2O$ in water and dilute to 1 liter. (If desired, this solution can be standardized against 0.1 N dichromate solution [4.903 g pure, dry $K_2Cr_2O_7$/ Liter] as follows: Pipet 25 mL of the dichromate solution into a 250 mL flask, add 25 mL of 1 N H_2SO_4 and 3 g KI. Mix, let stand 1 minute, then titrate with the thiosulfate solution, using starch as the indicator.)

<u>Note:</u> When analyzing the reducing sugars, make accurate dilutions of the 0.1 N thiosulfate to 0.005 N for the titration: 50.0 mL of 0.1 N thiosulfate, diluted to 1 liter.

3. STARCH INDICATOR SOLUTION: Make a suspension of 10 g soluble starch in ~50 mL of water. Slowly pour this suspension into 950 mL of boiling water. Let boil, with stirring, for 3-5 minutes longer, then cool. Add 1 g $ZnCl_2$ (or a crystal of thymol) as a preservative.

4. CALIBRATION CURVE: Dissolve 0.90 g pure anhydrous glucose in 100 mL 0.01 N H_2SO_4 and mix thoroughly. This is the "stock solution." Dilute exactly 5.0 mL of the stock solution to 100 mL with water. This is the "working solution." Carry out the sugar analysis with aliquots of 5, 4, 3, 2, 1 and 0 mL of the working solution, adding water to make a total volume of 5 mL in each tube. The copper reagent is then added and the analysis carried out as described under Procedure. The volume of 0.005 N thiosulfate required for each of the sugar-containing tubes is subtracted from that for the tube containing only water (the Blank). These values are then plotted against the respective percentages of glucose to give the calibration curve.

Note: The 0.005 N thiosulfate solutions and the diluted glucose solutions should be prepared just before use. The stock solution of glucose, the 0.1 N thiosulfate solution, and the starch indicator solution can be kept for many months in a refrigerator. The copper reagent does not require refrigeration.

Procedure for Analysis:

1. Make a 1:400 dilution of the soft drink to be tested.

2. Prepare "working solutions" of glucose as described above.

3. Pipet 5 mL of soft drink dilution in each of three 25x200 mm test tubes.

4. Pipet 5, 4, 3, 2 and 1 mL of the working solution into three test tubes each. Add sufficient water to make the volume exactly 5 mL.

5. Pipet 5 mL of water into each of three tubes for the Blank determination.

6. Add 5 mL of the copper reagent to each tube. Cover loosely with a square of aluminum foil.

7. Place tubes in boiling water bath and boil for 15 minutes, then cool in cold water. (Samples containing reducing sugars will have a dark brownish precipitate of Cu_2O. The Blank tubes will remain blue.)

8. Add 5 mL of 1 N H_2SO_4 (use graduated cylinder) to each tube and swirl well. A brown color and frothing will be noted as the iodine is liberated.

9. Transfer each tube into a separate flask or beaker for titration, carefully rinsing out the tube 2-3 times with distilled water.

10. Titrate each sample with the 0.005 N thiosulfate solution until the color is light yellow. Then add a few drops of the starch indicator solution and continue titrating until the blue-black starch-iodine color is gone. (Solutions may have a faint blue color due to unused copper reagent.)

11. Titrate each Blank sample the same way. This titration should require about 22 mL of the 0.005 N thiosulfate solution. Average the three values.

12. Average the three trials of each sample and subtract from the Blank value. This value is then used with the Calibration Curve to determine the percent of reducing sugar in the soft drink, taking into consideration the amount of dilution.

Concluding the Analysis:

When the amount of reducing sugars (assuming that these are glucose and fructose) and the amount of glucose have been determined, subtract the glucose value from the reducing sugars value, and this value should equal the amount of fructose present.

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