CHE2810: At-home Laboratory Series

Experiment 2: Analysing Starch and Sugar in Food

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Interpreting AOAC (Association of Analytical Communities) Official Method 925.38 for determining starch in fruit and fruit products – qualitative test

Why use AOAC methods?

AOAC methods are validated methods that laboratories can use to compare their experimental results with other scientists. These methods have been proven to be reliable and repeatable

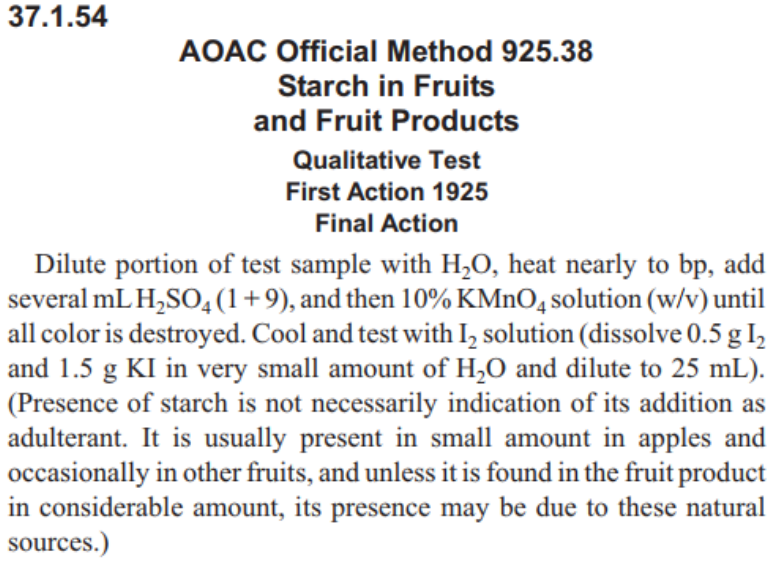
Safety Precautions

This experiment will involve handling iodine based chemicals (betadine) – if you are allergic to iodine it may be best to avoid this experiment.

1. **Read ALL instructions before commencing.**
2. **Ensure you have a clear space to manoeuvre while carrying out this experiment, to avoid slips, trips and falls.**

Methodology

For determining starch presence in fruit and fruit products a simple procedure is used.



37.1.54

AOAC Official Method 925.38
Starch in Fruits
and Fruit Products

Qualitative Test
First Action 1925
Final Action

Dilute portion of test sample with H_2O, heat nearly to bp, add several mL H_2SO_4 (1 + 9), and then 10% $KMnO_4$ solution (w/v) until all color is destroyed. Cool and test with I_2 solution (dissolve 0.5 g I_2 and 1.5 g KI in very small amount of H_2O and dilute to 25 mL). (Presence of starch is not necessarily indication of its addition as adulterant. It is usually present in small amount in apples and occasionally in other fruits, and unless it is found in the fruit product in considerable amount, its presence may be due to these natural sources.)

Figure 1 AOAC Method for determining starch presence in fruit and fruit products.

The method states that you should:

1. Use chemicals which are not readily available in the home and may not be safely used using typical PPE in the home (e.g H_2SO_4 and $KMnO_4$), so it is not ideal to do in this type of environment. We have an alternative.

2. To bypass this we are going to use a substance to test for starch presence that may be readily available at home, in pharmacies or in pet stores – Betadine® antiseptic liquid or spray.
3. Betadine contains Povidone Iodine, which is a a chemical complex of povidone, hydrogen iodide, and elemental iodine.
4. When iodine is in the presence of starch, it reacts to form a very deep blue-purple color. We all know that flour contains starch and thus it should appear blue-purple when iodine is present

Please watch the CHE2810 laboratory demonstration video to understand more how to do this experiment before doing it.

Equipment for Starch Test

Items in your home you will need are:

1. Betadine liquid or spray (can be purchased at pharmacy or some supermarkets)

2. Foods from around your kitchen – it will help if they are white to show the colour properly. Suggestions are:
    a. Flour
    b. Bread
    c. Potato
    d. Salt
3. Disposable plates

You will also need to use items from your laboratory kit that are not everyday items that you will find in the home. Versions of these items would be found in an analytical laboratory.

For this experiment you will need the following items from your lab kit:

Safety Glasses

Gloves

Figure 2 Laboratory kit items required for this experiment.

Check that you have all these items and then commence with your experiment. During the experiment, you will need to record data in order to determine starch presence in your food samples.

Procedure for Starch Test

1. Place samples of your foods on the paper plates so that they cover an area of approximately 7 x 7 cm each.
2. Carefully apply the betadine spray or liquid to one of your samples and immediately observe and record the colour. Take photos if you wish.
3. Repeat Step 2 in turn with all of your other samples.
4. If any of the samples turn a deep purple-blue colour, record these as containing starch.

Recording of Data on Starch Presence

Food Item Tested	Colour of sample on application of Betadine	Starch is Present? (Y/N)

Interpreting AOAC (Association of Analytical Communities) Official Method 959.11 Glucose in Sugars and Syrups Shaffer-Somogyi Micro Method

Why use AOAC methods?

AOAC methods are validated methods that laboratories can use to compare their experimental results with other scientists. These methods have been proven to be reliable and repeatable

Safety Precautions

This experiment is relatively low risk to carry out.

1. **Read ALL instructions before commencing.**
2. **Ensure you have a clear space to manoeuvre while carrying out this experiment, to avoid slips, trips and falls.**

Methodology

For measuring glucose content in sugars and syrups one of the procedures used is the following

44.1.23

AOAC Official Method 959.11
Glucose in Sugars and Syrups
Shaffer-Somogyi Micro Method
First Action 1959
Final Action 1960

A. Reagents

(a) *Shaffer-Somogyi carbonate 50 reagent, 5 g KI.*—Dissolve 25 g each of anhydrous Na_2CO_3 and potassium sodium tartrate·$4H_2O$ (Rochelle salt) in ca 500 mL H_2O in 2 L beaker. Add through funnel with tip under surface, with stirring, 75 mL of solution of 100 g $CuSO_4 \cdot 5H_2O$/L. Add 20 g $NaHCO_3$, dissolve, and add 5 g KI. Transfer solution to 1 L volumetric flask, add 250 mL 0.0167M KIO_3 (3.567 g dissolved and diluted to 1 L), dilute to volume, and filter through fritted glass. Age overnight before use.

(b) *Iodide–oxalate solution.*—Dissolve 2.5 g KI and 2.5 g $K_2C_2O_4$ in H_2O and dilute to 100 mL. Prepare fresh weekly.

(c) *Thiosulfate standard solution.*—0.005M Prepare daily from standardized stock 0.1M solution, 942.27 (*see* A.1.13).

(d) *Starch indicator.*—Rub 2.5 g soluble starch and ca 10 mg HgI_2 in little H_2O. Dissolve in ca 500 mL boiling water.

B. Determination

Pipet 5 mL solution containing 0.5–2.5 mg glucose into 25 × 200 mm test tube. Add 5 mL reagent, **(a)**, and mix well by swirling. Prepare blank, using 5 mL H_2O and 5 mL reagent. Place tubes, capped with bulb or funnel, in boiling water bath 15 min. Carefully remove tubes without agitation to running water cooling bath 4 min. Remove caps and add down side of each tube 2 mL KI–$K_2C_2O_4$ solution and then 3 mL 1M H_2SO_4 (56 mL/L). (Do not agitate solutions while alkaline.) Mix thoroughly to ensure that all Cu_2O is dissolved, and let stand in cold water bath 5 min, mixing twice during that time. Titrate with 0.005M $Na_2S_2O_3$, using starch indicator, **A(d)**. Subtract titration of test solution from that of blank and determine amount glucose in 5 mL solution from Table **959.11**.

Make control determinations with known amounts of glucose and apply corrections for any deviations from tabulated equivalents.

CAS-50-99-7 (glucose)

Figure 3 AOAC Method for determining glucose in sugars and sugar syrups

The method states that you should:

1. Use chemicals and make up reagents which are not readily available in the home and may not be safely used with typical PPE in the home.
2. "Pipet 5 mL solution containing 0.5–2.5 mg glucose into 25 x 200 mm test tube"
   - *2.1. This means that you would use a calibrated pipette or automatic pipettor to transfer 5 millilitres of your sample solution into a test*

tube. The method specifically states also that it should contain about 0.5-2.5 grams of glucose, so you may need to dilute your sample so this condition is met. ***You will also prepare your samples to be approximately this volume, and you will calculate how to dilute your samples so they do not fall outside the limits of measurement.***

3. "Add 5 mL reagent, (a), and mix well by swirling. Prepare blank, using 5 mL H2O and 5 mL reagent."

3.1. This means that you add the first reagent to your samples. Preparing a blank in this instance, means preparing a sample that you know to contain no glucose at all. ***You will also prepare a blank in your experiment.***

4. "Place tubes, capped with bulb or funnel, in boiling water bath 15 min. Carefully remove tubes without agitation to running water cooling bath 4 min. Remove caps and add down side of each tube 2 mL KI–$K_2C_2O_4$ solution and then 3 mL 1M H_2SO_4 (56 mL/L). (Do not agitate solutions while alkaline.) Mix thoroughly to ensure that all Cu_2O is dissolved, and let stand in cold water bath 5 min, mixing twice during that time. Titrate with 0.005M $Na_2S_2O_3$, using starch indicator, A(d). Subtract titration of test solution from that of blank and determine amount glucose in 5 mL solution from Table 959.11. Make control determinations with known amounts of glucose and apply corrections for any deviations from tabulated equivalents."

4.1. This means that the first reagent needs heat to catalyse the reaction and the following reagents are needed after the heating phase. To 'titrate' means to slowly add small volumes of the reagent to your sample until a colour change occurs.

5. **Because many of the chemicals in the AOAC method are not readily or safely available for home use, you will bypass this by using the glucose test strips in your laboratory kit.** The reaction that the glucose in your samples will have with the strips will drive a colour change on the strip. The colour shown will indicate the level of glucose present.

Please watch the CHE2810 laboratory demonstration video and read the procedure below to understand more how to do this experiment before doing it.

Equipment and materials for Glucose Test

Items in your home you will need are:

1. Sugary liquids and foods for your food samples. This may include the following:
    a. Soft drink
    b. Fruit juices
    c. Juice squeezed from fresh fruit
    d. Whole fruit with a fresh cut surface that is still wet
2. A stopwatch or stopwatch function on your phone/computer to time the tests
3. Deionised water sold as ironing water in the supermarket (normal tap water may be ok as it should not often contain sugars.
4. A teaspoon for mixing.

You will also need to use items from your laboratory kit that are not everyday items that you will find in the home. Versions of these items would be found in an analytical laboratory.

For this experiment you will need the following items from your lab kit:

Safety Glasses

Gloves

Glucose Test Strips

Plastic droppers

Figure 4a) Laboratory kit items required for this experiment.

25 mL measuring cylinder

250 mL beakers

Figure 4b) Further laboratory kit items required for this experiment

Check that you have all these items before commencing with your experiment. During the experiment, you will need to record data in order to determine glucose content in your food samples.

Calculating dilution volumes

Some samples may need to be diluted so they fall into the measurement range of the glucose test strips. We have provided a simple dilution ratio in the experimental procedure, but if you wish to calculate a more accurate dilution, please use the calculation method below.

1. Where possible first check the sugar content of your liquid samples – this can be read from their nutrition panel, an example of which is shown below.

2. Convert the sugar content from the label into mg/dL so that it can be measured against the test strip scale (milligrams per decilitre – a decilitre is one-tenth of a litre or 100 mL). The way to do this is as follows:
    a. Record the grams of sugar per 100 mL – this is the grams of sugar in 1 dL.
    b. Convert this number of grams into milligrams by multiplying by 1000.
    c. Using the example adjacent, we would get 13.4 grams x 1000 = 13,400 mg/dL

3. Determine if your sample fits in the test range of the glucose strip scale shown below – the upper limit is 990 mg/dL (numbers in black). If your sample does not fit, you can calculate in Step 4 how to dilute it.

4. We will use the juice example in Step 2 and aim for an approximately mid-range concentration on the 0-990 mg/dL scale shown for the glucose test strips.

$$C_1 x V_1 = C_2 x V_2$$

Where:
C_1 = the initial high concentration of your liquid (13,400 mg/dL in this case)
V_1 = initial volume of your liquid (we will use 5 mL here, same as in the AOAC method, which equals 0.05 dL – simply divide by 10 to convert)
C_2 = desired diluted concentration of your liquid (we will use 450 mg/dL here)
V_2 = the final volume of your liquid including added water for dilution

V_2 is our unknown and we need to determine this to calculate how much deionised (ironing water) we need. To do this, the formula is rearranged to isolate V1 and then we can substitute in our values as follows.

$$V_2 = \frac{C_1 x V_1}{C_2} = \frac{13{,}400 \text{ x } 0.05}{450}$$

= 1.48 dL = 148 mL (simply multiply by 10 to convert)

This means that to dilute the sample to an appropriate level you will need to add 1.48 – 0.05 dL to the 5 mL of sample. Which is 1.43 dL or 143 mL.

Procedure for Glucose Test

1. First dilute any samples that fall outside the glucose strip measurement range. To make it easier your total diluted volume can just be 150 mL (containing 5 mL of juice) as we are doing an approximate dilution so your sample can be tested by the strips. This dilution will work for most of your samples and keep them in the measurement range if they contain about 5-30 g of sugars per 100 mL. For anything higher than this you may need to dilute twice, i.e. Dilute your diluted sample using the same procedure.
    a. So to dilute your liquid samples, measure 5 mL of your liquid sample using the measuring cylinder and pour into a clean dry 250 mL beaker. Using the same measuring cylinder, measure out 145 mL of ironing (or tap) water and add to the beaker containing your sample and mix with a spoon. You will need to use the measuring cylinder a few times to do this which is good as it will help to rinse any remaining sample residue into the beaker.
2. To test your samples do the following:
    a. Put on your gloves.
    b. Pick up the glucose strip via the non-test end (it will be flat with no raised surface)
    c. Hold the test strip over a plate or paper towel and using the plastic dropper wet the test end of the test strip and time for 2 minutes.
    d. When the time is up, check the colour of the test strip against the scale shown below to determine the glucose concentration in your diluted food sample and record this in the Results table. If it is between 2 values, take the mid-point.

3. Back-calculate the original glucose concentration and record in the results table.
    a. To calculate the actual food sample concentration use the following formula (another re-arrangement of the dilution formula)

$$C_1 = \frac{C_2 x V_2}{V_1}$$

Where this time:
C_1 is the true sample concentration,
C_2 is the diluted concentration measured with the test strip,
V_1 is the 5 mL of sample
V_2 is the diluted volume (150 mL in this case)

Recording of Data on Glucose content

The data to record in the table on the next page are:

Labelled sugar content from product nutritional panel (if no panel, just leave blank)
Glucose concentration of the diluted sample according to the glucose strip test
Actual glucose concentration in the original sample using the formula in Step 3 of the experimental procedure.

Table 1: Table for recording experimental data

Sample Name / ID	Repeat Number	Labelled sugar content (g / 100 g)	Glucose concentration from test strip (mg / dL)	Actual glucose concentration accounting for dilution	Comments