LAB 28: VITAMIN C:
ANALYSIS AND HEAT EFFECTS

PURPOSE: To investigate compare the vitamin C content in a variety of citrus juices.
To determine the effect of heat upon vitamin C content.

SAFETY CONCERNS:
Always wear safety goggles.

VITAMINS:
Vitamins are organic compounds required as cofactors or coenzymes for certain enzymes. However,
vitamins are not synthesized by the body and must be provided in our diet. The deficiency of a vitamin in
the diet can affect the activity of an enzyme, bringing about a deficiency disease.

Vitamin C promotes absorption of iron so a lack can cause scurvy (scorbutus), a condition characterized
by bleeding gums and anemia.
A deficiency in vitamin A is associated with night blindness.
A diet low in vitamin D can cause rickets in children.
A deficiency in B₁₂ can lead to anemia.

Because vitamins B and C have polar groups, they are soluble in water. The body usually excretes any
excess in these vitamins. Vitamins A, D, E, and K are nonpolar, fat-soluble vitamins.

VITAMIN C:
The body uses vitamin C, also called ascorbic acid, to fight infection and repair damaged tissue.
Ascorbic literally means “without scorbutus”. Vitamin C is found in many fresh fruits and vegetable
including oranges, grapefruit, broccoli, lettuce, and green peppers. Because vitamin C is a reducing agent
(also called anti-oxidant), we can detect it by its reaction with iodine, I₂. Vitamin C is oxidized and the I₂
is reduced. The indicator used in this reaction is a starch solution. When iodine (I₂) is present, the starch
turns a deep-blue color. However, when vitamin C is in the solution, the I₂ is reduced to iodide (I⁻), and
the starch does not form the blue-black color.

Vitamin C (L-ascorbic Acid)

Antioxidant.
Essential to Hydroxylation
of collagen.
(Collagen = structural protein
of connective tissue in skin, bone,
cartilage, etc.)
Found in: citrus fruits, (oranges, lemons, limes, grapefruit...,)
berries, broccoli, cabbage, peppers, tomatoes.
Lack causes scurvy:
In this experiment, we will determine the number of milligrams of vitamin C that react with 1 mL of iodine reagent. Then using starch as an indicator, we will determine the milligrams of vitamin C present in a sample of fruit juice or drink.

\[
\text{Starch (amylose) + I}_2 \rightarrow \text{Deep blue colored complex}
\]

Vitamin C loses its activity when it is heated. One sample of juice will be heated to determine the amount of vitamin C destroyed by heat.

**PROCEDURES:**
**ACTIONS:**

I. **STANDARDIZATION OF VITAMIN C:**

A. **Set Up:**

1. Accurately weigh out 100 mg (0.100 g) of ascorbic acid (vitamin C) and transfer it to a 100 mL Volumetric flask.\(^1\)
   Fill about ½ full with deionized water and swirl to dissolve completely. Then fill the volumetric flask to the 100 mL mark with more water and mix well.

2. Pipet out 10 mL of the ascorbic acid solution and place in a 250 mL Erlenmeyer flask.

3. To the flask of ascorbic acid add and mix in
   - 50 mL of deionized water\(^1\),
   - 6 drops of 6 M HCl and
   - 20 drops of 1 \% starch indicator.\(^3\)

4. Place a 50 mL burette in a burette clamp on a ring stand. Carefully fill the burette just above the zero mark with a 0.005 M iodine solution.\(^4\)

5. Drain the iodine down exactly to the zero mark. Record the initial level of the iodine solution.\(^5\)

B. **TITRATION:**

6. Place a white paper under the sample flask to better see a color change and then begin adding the iodine solution drop by drop from the buret to the vitamin C solution with constant stirring until the solution just turns a deep-blue/black color. (This endpoint is reached after all of the vitamin C has been oxidized and the next drop of iodine solution is not reduced.)

7. Record the final reading of the iodine solution in the buret and calculate the volume of iodine solution used in the titration.

8. Calculate the mass (mg) of vitamin C that reacts with 1 mL of iodine solution.\(^6\)

**NOTES:**

1. You may instead weigh out 10 mg solid ascorbic acid on an analytical balance. If 10 mg of solid ascorbic acid is used then put it directly into an Erlenmeyer flask and proceed to step 3.

2. The 6 drops of 6M HCl may be substituted with 2 mL of 0.1 M HAc (acetic acid).

3. The 20 drops of 1 \% starch solution may be substituted with 7 drops of 3 \% starch solution. Starch is used as the indicator of a completed reaction. When the reaction is over and all Vit C has reacted with the added I\(_2\) then any excess unreacted iodine (I\(_2\)) present will fit into the coiled amylose of starch and turn deep blue. When you see the deep blue color you know that there is no longer any Vit C left to react with the added iodine.

4. Overfilling the burette and then draining both rids the burette tip of air bubbles and assures an exact zero starting point.

5. If it is not practical to fill the burette to zero then record exactly the starting iodine level so that you can keep track of how much is used. Do not let the level of Iodine solution go below the lowest mark on the burette or there will be no way to indicate mLs used. If you need to add more solution to the burette simply keep track of the total used.

\[\text{(mg Vit C in tablet)} = \frac{\text{mg Vit C}^\ast \text{oxidized by 1 mL I}_2 \text{ solution}}{\text{(mL I}_2 \text{ sln)}}\]

\(^6\)needed for calculation of Vit C in foods in Part II.
II. VITAMIN C CONTENT OF FOODS:

A. Sample Preparation:

Fruit Juices:
1. Obtain a 20 mL sample of fruit juice\(^7\) or fruit drink\(^8\) for vitamin C analysis and put into a 125 mL Erlenmeyer flask. Record the type of juice or drink.\(^9\)

2. To the flask of juice add and mix in
   - 25 mL of deionized water,
   - 6 drops of 6 M HCl and
   - 20 drops of 1 % starch indicator.\(^3\)

Vegetables:
3. Puree 10.0 g of a vegetable\(^10\) in a blender, juicer or large mortar & pestle with 25 mL of deionized water.

4. Filter the puree through cheesecloth and place in a 125 mL Erlenmeyer flask.

5. Add and mix in
   - Enough deionized water to give a total volume of 50 mL,
   - 6 drops of 6 M HCl and
   - 20 drops of 1 % starch indicator.\(^3\)

B. Titration:
6. Record the initial reading of the level of iodine solution in the buret. Refill if needed.

7. Place a white paper under the sample flask to better see a color change and then begin adding the iodine solution **drop by drop** from the buret to the vitamin C solution with constant stirring until the solution just turns a deep-blue/black color.

8. Record the final buret reading for the level of iodine and calculate the volume of iodine solution used to reach the endpoint of the titration.

9. Calculate the mg of vitamin C in the fruit juice sample.\(^11\) Use the value of mg of vitamin C per 1 mL of iodine solution obtained in Part I.9.

10. Determine how many mLs or grams of this food should be consumed in order to meet the minimum daily requirement (RDA) of 75 mg/day.

11. Repeat the titration with another sample.

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\(^7\)This experiment works best if the juice or food sample tested is colorless or has a light color. Orange juice, grapefruit juice, white grape juice, HiC or Snapple etc. can be used.

\(^8\)Powdered drinks such as Tang or Kool-Aid can be used. If using a powdered drink weigh 1.0 g of the powder and mix it with 50 mL of deionized water in step 2 (rather than 25 mL) and then proceed as with other juices.

\(^9\)If the juice has a lot of pulp (fiber), pour the juice through cheesecloth that covers a funnel. If suspension particles go through, use two layers of cheesecloth. Filter enough juice so that you end with 20 mLs of pulp free juice to test.

\(^10\)Vegetables such as broccoli or spinach can be used.

\(^11\)\[ \text{\textit{mL I}_2 \text{ sln x (mg VitC)} \times (1 \text{ mL I}_2 \text{ sln})} \]

\[ = \text{mg Vit C} \]

*from calculation in Part I.8.*
III. Heat Destruction of Vit C:
1. Choose a light colored juice in Part II that had a high vitamin C content and place 75 mL of the juice into a 250 mL Erlenmeyer flask.

2. Cover the top of the flask with a watch glass and place the flask of juice on a hot plate. Heat the juice but do not allow it to boil. **Do not allow the heat to be high enough that the water boils away.** Note the time.

3. After about 5 minutes of heating pipette out 20 mLs of juice into another Erlenmeyer flask for testing. Place this sample in an ice-water bath to cool it.

4. Continue heating the remaining sample, continuing to take care not to heat it so hot that it boils away or condenses.

5. After another 10 minutes of heating (so that would be 15 minutes total) pipette out another 20 mLs of juice into another Erlenmeyer flask for testing. Place this sample in an ice-water bath to cool it.

6. To each cooled “5 minute” and “15 minute” juice samples add 6 drops of 6M HCl and 20 drops of 1% starch indicator.

7. Fill a buret with iodine solution and record the initial level.

8. Titrate each solution with iodine solution to an **identical** deep-blue/black endpoint. Record the final level of iodine solution and calculate the volume of iodine solution used in each titration.

9. Calculate the mg of vitamin C present in each of the heated samples. Graph your results.

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You want to achieve the same color endpoint so that you can compare Vit C values. If one sample is darker than the other you may have added different amounts of Iodine.
LAB 28: VITAMIN C: 
PRE LAB EXERCISES: 

1.____ The metabolic role of vitamin C in the body is to
   A. fight infection.                   B. repair damaged tissues.
   C. act as an antioxidant.            D. aid in the formation of collagen.
   E. promote absorption of iron.       F. More than one but not all of these.
   G. All of these.

2.____ Starch is used as an indicator in this experiment because
   A. it is an excellent reducing agent.
   B. It turns blue in the presence of free iodine.
   C. it turns blue in the presence of iodide ion.
   D. it prevents ascorbic acid from decomposing.
   E. all of these.

3.____ If you analyzed a “50 mg” tablet of vitamin C **accurately** and discovered that it contained only 30 mg of ascorbic acid, you would suspect that
   A. it was contaminated.
   B. it may have been old.
   C. it may have been stored in a cool room.
   D. the binder in the tablet caused some of the ascorbic acid to decompose.
   E. all of these.

4.____ When the titration endpoint is reached in this experiment, the solution in the Erlenmeyer flask will
   A. become deep blue.                  B. become colorless.            C. begin to foam.
   D. form a precipitate.               E. all of these

5.____ Show the balanced equation for the reaction of Vitamin C with Iodine (I₂): (Use structures not formulas for Vitamin C.)

6.____ Determine the MW of vitamin C. Show your work and circle your answer.

7.____ If a 20mg sample of Vitamin C was titrated with a 0.005 M iodine solution how many mLs of I₂ solution would be expected to reach the endpoint? **Show your calculations.**
### I. Standardization of Vitamin C:

<table>
<thead>
<tr>
<th>A. Step 1. Mass of Vit C used</th>
<th>Trial 1</th>
<th>Trial 2 (If needed)</th>
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<td>(from label)</td>
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<tr>
<th>B. Step 5. Initial buret reading</th>
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<th>C. Step 7. Final buret reading</th>
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<tr>
<th>D. Volume of I(_2) sln used</th>
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<tr>
<th>E. Step 8.</th>
<th>Show calculations:</th>
<th>Show calculations:</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg Vit C oxidized (A)</td>
<td>= mg Vit C</td>
<td>= mg Vit C</td>
</tr>
<tr>
<td>mL iodine solution (D)</td>
<td>1mL I(_2) sln</td>
<td>1mL I(_2) sln</td>
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### II. Vitamin C Content of Foods:

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<tr>
<th>F. Food Sample:</th>
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<tr>
<th>G. Sample size</th>
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<tr>
<td>in mLs or g’s</td>
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<tr>
<th>H. Step 6. Initial buret reading</th>
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<th>I. Step 8. Final buret reading</th>
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<th>J. Volume of I(_2) sln used</th>
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<tr>
<th>K. Step 9.</th>
<th>Show calculations</th>
<th>Show calculations</th>
<th>Show calculations</th>
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<tbody>
<tr>
<td>mg Vit C in the sample = _mL I(<em>2)</em> x _mg VitC = mL or g sample / mL I(<em>2)</em> <em>I \times E</em> G</td>
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<table>
<thead>
<tr>
<th>L. Step 10. Total mLs or g’s to meet RDA</th>
<th>Show calculations</th>
<th>Show calculations</th>
<th>Show calculations</th>
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</thead>
<tbody>
<tr>
<td>(from your data how much juice/food would you need to consume to reach the RDA of Vit C?)</td>
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III. Heat Destruction of Vitamin C:

<table>
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<tr>
<th>Sample:</th>
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<tbody>
<tr>
<td>Before Heating (New or from part II)</td>
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<tr>
<td>A. Heated 5 min</td>
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<tr>
<td>B. Heated 15 min</td>
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<table>
<thead>
<tr>
<th>Step 6 Initial buret reading</th>
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<tbody>
<tr>
<td>Step 7 Final buret reading</td>
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<tr>
<td>Volume of I₂ sln used</td>
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</table>

| Step 8 |
| mg Vit C in the sample |
| Show calculations & units |
| Show calculations & units |

| mg Vit C lost from beginning |
| 0 |

Graph: mg Vit C vs heating time:

Conclusions/Explanation/Analysis: Does heating affect the vitamin C content of a fruit juice? Explain your observations and make a conclusion. Discuss any errors.

1. Based on your results of heating for 5 minutes, calculate the rate of decomposition of mg Vit C per minute when boiling a fruit or vegetable? Show your calculation.

2. From your graph, how long would you have to heat a sample of juice containing 10 mg Vit C before the Vitamin C completely decomposed? Show calculations.
LAB 28: VITAMIN C:  
NAME____________

RELATED EXERCISES:
DATE____________

1. ___ What are the symptoms associated scurvy caused by a lack of vitamin C?
   A. rickets
   B. night blindness.
   C. bleeding gums.
   D. More than one of these is correct.

2. ___ The word “ascorbic” means
   A. aspirin-like
   B. without scurvy
   C. without scruples
   D. ask or bic
   E. None of these

3. ___ In the determination of heat destruction of Vitamin C Part III, the starch indicator was
   added to the ascorbic acid solution after it was heated rather than before heating. This
   was because
   A. Ascorbic acid would denature the starch causing it to coagulate.
   B. Heating in water would cause starch to hydrolyze into glucose which would then not
      coil around excess iodine.
   C. Starch might boil away and be lost before the titration was begun.
   D. More than one of these is correct.

4. Show the equation for the hydrolysis of Vitamin C in boiling water. (Use structures not
   formulas for Vitamin C.)
Lab 28B: Vitamins: Name ____________________________

**Night Blindness:** Video Clip: Rx for Survival: The Heroes

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<th>Vitamin Deficiency =</th>
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<td>**Name of Researcher =</td>
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<td>**Populations Affected =</td>
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<tr>
<td>**Ramifications of this vitamin deficiency =</td>
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**Conclusions/Explanation/Analysis:** What have you learned or concluded?

**Skin Problems in Orphans:** See attached article

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**Conclusions/Explanation/Analysis:** What have you learned or concluded?
Decoding a Turn-of-the-Century Mystery

Offer someone sow bellies and cornmeal grits for dinner tonight, and you'll probably find yourself eating alone. But in the deep South less than 100 years ago, these foods were the staple diet that's thought to have caused an outbreak of a niacin-deficiency disease called pellagra.

Characterized by a progressive decline that often starts with itchy, red skin, moves on to diarrhea and depression and ends in death, pellagra afflicted more than 100,000 people in 1914.

The incidence was of such alarming proportions that the U.S. Surgeon General called for a special investigation of 'one the knottiest and most urgent problems of the present time.' Dr. Joseph Goldberger was asked to investigate.

An experiment involving orphans in Jackson, Mississippi, who had pellagra soon provided clues to the mystery. Just a few days after doctors added milk, meats and eggs to the corn grit diet of the children, their pellagra disappeared.

To confirm dietary deficiency as a cause, another study was conducted, this time involving convicts. When pellagra-free prisoners agreed to eat nothing but sow bellies, corn grits, gravy and fried mush for five months, nearly all developed pellagra.

To eliminate lingering doubts that pellagra might be an infectious disease, Goldberger launched, still another study involving convicts. The prisoners, and the experimenters themselves, were injected with blood from people with pellagra or were exposed to their nasal or throat excretions. When the subjects didn't come down with the disease, researchers realized that pellagra could not possibly be infectious.

True Grits Not Enough

Although researchers soon figured that poor diet was causing pellagra, it wasn't until 1937 that researchers pinpointed the exact source of the problem. Corn not only contains a form of niacin, a B vitamin, that the body cannot easily use but also can create an amino acid imbalance. Amino acids are the building blocks of protein, the stuff of which the body is made.

Eating corn as part of a well-balanced diet is not a problem, but a diet that consists almost exclusively of corn and corn products is devastating. In fact, the turn-of-the-century pellagra tragedy led to the fortification of flours and cereals with niacin. As a result, pellagra is now rare.

A few people still get pellagra, however, for reasons that have nothing to do with eating corn. Alcoholics and individuals with severe gastrointestinal problems often have difficulty getting enough niacin. Even then, diagnosing pellagra is not easy. The early symptoms, such as reddening of the skin, cracked lips, weight loss, tiredness, confusion and mild diarrhea, can be subtle.

Niacin to the Rescue

Fortunately, the effects of early-stage pellagra are easily reversed. "In general, the main therapy is to get patients back on a healthy diet and, in the case of alcoholics, to get them off of their alcohol and on to some kind of niacin supplementation," Drs generally prescribe niacinamide, a form of niacin that's known not to produce undesirable side effects.

The amount of niacinamide a person with pellagra should take really depends on the condition of the individual and should be determined by a doctor.

Some of the best food sources of niacin are chicken breast, tuna and veal. The Daily Value for niacin is 20 milligrams.