VERSUS

Dr. Stewart

Mike Dubien
Biotechnology 2
February 2015
Block 2
Abstract

Companies who produce nutritional supplements and medicines want to do every test that they possibly can in order to assure 100% satisfaction to the pharmacy purchasing their product. A key component of testing the effectiveness of a nutritional supplement would be to examine how the digestive process impacts the supplement’s ability to properly benefit one’s body. Vitamins are nutritional supplements that people take every day, but there is always debate on whether it is more beneficial to take daily vitamins or to eat foods rich in essential vitamins.

A 300 mL. Stomach acid solution was created using 150 mL. Of a 0.5% Pepsin solution and 150 mL. Of a 4 mL. Concentrated hydrochloric acid/250 mL. deionized water solution. In six 150 mL. beakers, 50 mL. of the stomach acid solution was transferred into each beaker using a serological pipette. Each beaker had a vitamin-rich food, a regular vitamin, or a fast-acting vitamin. The beakers were left in a 37°C water bath for a six hour period to simulate digestion in the stomach.

A starch-iodine solution test was used to test Vitamin C apparent release levels in the beaker and the Vitamin B-12 release levels were tested using the spectrophotometer. Conclusions show that the soybeans had similar Vitamin C levels when compared to the Vitamin C chewable. The fast-acting B-12 vitamin showed the highest level of Vitamin B-12, which shows fast-acting vitamins are true to their label.

Background

There are millions of Americans today that live with vitamin deficiencies. In fact, the struggle with vitamin deficiency can be traced back as far as to the 1700s when English naval captain and surgeon James Lind took a trip with 350 sailors. (4.) Lind came to the conclusion that eighty of his 350 sailors had gotten scurvy on their voyage because of a vitamin C deficiency. He fed the sailors who had became ill lemons and limes because they would be the fruits that would last the longest as they were on their ten week voyage, and the lemons and limes cured their scurvy because of their high levels of vitamin C. This was the first instance in history showing the importance of getting the proper amount of vitamins in a daily diet. Another study was done in 1929 by Dr. Frederick Hopkins showing the importance of vitamins. (4.) Hopkins gave lab rats a weak diet of simply fats, carbohydrates, proteins, and only minerals without vitamins. The lab rats showed signs of malnutrition as they had difficulty growing as well as becoming ill often. These two discoveries prove that vitamins play a huge role in having a healthy diet.
Vitamins are organic compounds found in food and/or nature that are needed by the human body to maintain its performance. People around the world take vitamins for three reasons: To treat a vitamin deficiency, to prevent the development of a vitamin deficiency, or to reduce the possibility of getting a disease. (5.) A vitamin deficiency can be treated in many ways, but the most common ways are by taking an oral supplement, injection of a needed vitamin, or a change in one's diet. (5.) People most commonly take their vitamins by taking oral supplements that can be found at their local supermarket. Two of the most common vitamins people have deficiencies with are vitamin B12 and vitamin C, which will be the focus of this experiment.

Vitamin B12, also known as cobalamin, helps maintain healthy nerve cells and red blood cells as well as contribute to making DNA in the body. (5.) Vitamin B-12 is also bound to protein and the hydrochloric acid in the stomach releases B-12 from protein during digestion. (5.) Once it has been released from the protein, B-12 binds to intrinsic factor before it is absorbed into the bloodstream. Foods high in this vitamin include fish, milk, milk products, eggs, meat, poultry, and breakfast cereals. A vitamin B-12 deficiency can result in pernicious anemia, celiac disease, and other gastrointestinal disorders. Most diseases resulting from a B-12 deficiency happen because of the absence of intrinsic factor.

Vitamin C is important to have in one's diet because of its effect on reducing the risk of having heart disease and cancer. (4.) Vitamin C is claimed to play a key role in an important protein in skin, cartilage, ligaments, tendons, and bones called collagen. It also is a powerful antioxidant that protects cells from disease and strengthens the immune system. Foods high in vitamin C include citrus fruits such as lemons, limes, and oranges.

Foods high in certain vitamins have been argued to be more beneficial to have more of in your diet as opposed to taking oral vitamins because these foods contain fiber as well as other macromolecules that vitamins do not contain. In this experiment, a solution that mimics the stomach acid will be created referencing an experiment that is identified in the bibliography. Using hydrochloric acid and pepsin, a stomach acid solution will be created. (2.) Pepsin is an enzyme naturally occurring in stomach acid that breaks down proteins as they are digested by the human body. (3.) The pH will be tested multiple times using pH paper until the proper pH of the solution matches that of stomach acid, which is a pH of 1. (1.) Six beakers will be used, and in three of the beakers vitamin B12 will be observed. In the other three beakers vitamin C will be observed. In each set of beakers specific to their vitamin, one beaker will contain a food rich in that vitamin, one will contain a normal vitamin, and the other beaker will contain a fast-acting vitamin. The observation period will be 6 hours, and the beakers will be placed in a 37°C water bath to further mimic the conditions of the stomach.

**Methods**

**Risk and Safety:**

- The MSDS was referenced for HCl and Pepsin to become aware of the level of hazard that they come with.
- Proper PPE will be worn throughout the duration of this experiment: Safety Glasses, Lab Coat, Gloves, Plants, and Closed-Toed Shoes
- All handling of the HCl was done in the fume hood to avoid inhaling any toxic fumes
**Initial Procedure:**

1. Take proper measures of sanitation. Due to HCl being a small hazard of a chemical, safety glasses, gloves, close-toed shoes, pants, and a lab coat should be worn as proper PPE.

2. Make the Simulated Stomach Acid Solution referencing the following recipe:
   - Dilute 4 mL. Concentrated HCl in 250 ml. \( \text{dH}_2\text{O} \) in a 500 mL. graduated cylinder **in the fume hood**
   - Dilute 1 g. Pepsin enzyme lab powder in 200 mL. \( \text{dH}_2\text{O} \) in another 500 mL. graduated cylinder
   - Pour 150 mL. of each solution into a separate 500 mL. graduated cylinder to make 300 mL. of Simulated Stomach Acid Solution

3. Using the pH paper, measure the pH of the solution to assure its pH is 1.

4. Pour 50 mL. of the Simulated Stomach Acid Solution into each of the six 150 mL. lab beakers.

5. Using the weigh balance weigh 15 g. Part-Skim, Low Moisture Mozzarella, and 15 g. Raw Edamame Soybeans (Soybeans must be squeezed out of their pods when being weighed)
   - Be sure to tare the weigh paper before weighing the foods to assure proper measurements
   - After weighing each food, place the mozzarella in one plastic bag and the soybeans in another and mash the foods up in their respective bags (Shows chewing) **BE CAUTIOUS OF THE BAG BREAKING!**

6. Label each of the 6 150 mL. lab beakers with a letter corresponding to the following:
   - A. Part-Skim Milk Mozzarella Cheese
   - B. 1000 MCG **Fast-Acting** Vitamin B12 (Soft gel)
   - C. 1000 MCG Timed-Release Vitamin B12 (Tablet)
   - D. Raw Soybeans Out-of-Pod
   - E. 500 MG Vitamin C (Caplet)
   - F. 500 MG **Fast-Acting** Vitamin C (Chewable)

7. Place each of the digestive substances into their corresponding beakers and place the beakers in the 37° water bath to further simulate the conditions of the human stomach for 6 hours to mimic the digestion period in the stomach. (Take a “Before” picture)

**Vitamin C Test:**

- Make 40 ml. of a starch solution
  - 40 ml. \( \text{dH}_2\text{O} \)
  - 5 g. Baking Soda
- Combine baking soda and water in a small beaker and vortex until the baking soda has gone into solution.
- While vortexing the starch solution, make the positive control solution for Vitamin C and put the control solution in a small beaker
  - 50 mL. Simulated Stomach Acid Solution
  - .5 g. Ascorbic Acid (Molecular form of Vitamin C)

- After 3 hours of being in the water bath, pipette 1 mL. of each of the following beakers into separate test tubes: Beaker D, E, F as well as 1 mL. from the positive control solution for Vitamin C into the fourth beaker

- Once 1 mL. of each solution has been put into its perspective test tubes, label the test tubes and add 10 drops of starch solution to each test tube using a rubber pipette
  - As the starch solution is added be sure to stir the test tube

- After 10 drops of starch solution has been added to all test tubes, one test tube at a time add iodine drop by drop until the solution in the test tube turns black
  - **Keep track of the number of drops of iodine for data**

**Explanation**

As iodine is added drop by drop to each one of the test tubes, it first reacts with the ascorbic acid, a molecular form of vitamin C, present in the solution. Once the ascorbic acid has been fully reacted with, the iodine then reacts with the starch solution which results in a black color. **Therefore, the more iodine added to initiate the reaction with the starch solution, the more ascorbic acid present, and the more vitamin C available.**

**Vitamin B-12 Test**

- After the six hour digestion period has completed take beakers B and C out of the water bath, and have the remaining stomach acid close by.

- Make the control solution by cutting a B-12 soft gel open and the squeezing the gel into a test tube containing 1 mL. of stomach acid.
  - Vortex the solution until everything is in solution

- Gather four cuvettes and a cuvette rack, and label the cuvettes and test tubes so they correspond with each other.
  - Be sure the label is placed towards the top of the cuvette so it does not interfere with the spectrophotometer reading

- Once everything has been labeled, pipette 1 mL. of each solution into their respective cuvettes

- Bring the cuvette rack over to the spectrophotometer and set the spectrophotometer to a 360 nm. Wavelength
- Blank the spectrophotometer by placing the cuvette with only stomach acid inside and read the sample. The reading should come up as 0.00
  - **Before putting any cuvette in the spectrophotometer, use a kimwipe to wipe the outside of the cuvette to ensure there is no liquid/substance present and that the reading is accurate.**
- After blanking the spectrophotometer, read the other three cuvettes and record the OD360 absorbencies in decimal form.
  - For the two cuvettes containing the test solutions, use a percentage conversion by dividing the OD360 absorbency by the OD360 absorbency of the positive control solution. This will calculate which of the two vitamins was fast-acting.

**Explanation**

Cobalamin is a chemical form of Vitamin B-12. According to its absorption spectrum, cobalamin has its highest color absorbency at light wavelength of 360 nm. The spectrophotometer shines light at this wavelength through the cuvette and is transmitted through everything except for cobalamin, which is absorbed. Therefore, the amount of available cobalamin in the solution is absorbed, and this amount is converted into a decimal.

**Results:**

**Vitamin C Results**

<table>
<thead>
<tr>
<th>Digestive Substance</th>
<th>Halfway Test</th>
<th>Full Time Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Control</td>
<td>75</td>
<td>56</td>
</tr>
<tr>
<td>Edamame Soybeans</td>
<td>31</td>
<td>52</td>
</tr>
<tr>
<td>Vitamin C Caplet</td>
<td>132</td>
<td>157</td>
</tr>
<tr>
<td>Vitamin C Chewable</td>
<td>34</td>
<td>59</td>
</tr>
</tbody>
</table>

**Vitamin B-12 Results**

<table>
<thead>
<tr>
<th>Digestive Substance</th>
<th>Cobalamin Absorbency (OD360)</th>
<th>Percent Conversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank (Stomach Acid)</td>
<td>0.000</td>
<td>0%</td>
</tr>
<tr>
<td>Positive Control</td>
<td>2.770</td>
<td>100%</td>
</tr>
<tr>
<td>Timed-Release B-12</td>
<td>0.302</td>
<td>11%</td>
</tr>
<tr>
<td>Fast-Acting B-12</td>
<td>0.635</td>
<td>23%</td>
</tr>
</tbody>
</table>
**Conclusion**

The goal of this experiment was to imitate digestion in the human stomach. The levels of vitamin C were tested in three of the six beakers (D, E, and F) in order to determine the benefits of taking a daily vitamin versus eating a diet of vitamin-rich foods. The levels of vitamin B12 were tested in two of the other three beakers (B, and C) in order to compare the differences between fast-acting vitamins and regular vitamin supplements while they are digested by the gastric juice in the gastric juice. Millions of people around the world have vitamin deficiencies, and this health issue can be dated as far back as the 1700s when sailors were getting a disease called scurvy that results from a vitamin C deficiency. From a business/consumer standpoint, many people purchase vitamins from themselves and for their young children, and companies creating these supplements would need to know how their supplement survives the digestive system starting with the stomach.
Evidence from the vitamin C Test concludes that the Edamame soybeans, a vitamin-rich food, had nearly as much vitamin C as the vitamin C chewable, which is commonly given to children at young ages. Although children may not prefer to eat vitamin-rich vegetables such as soybeans, they have nutritional values such a protein and fiber that taking a daily vitamin will not improve. These pediatric chewable vitamins also contain a smaller dosage than a regular vitamin would; however the chewable used in this experiment had the same dosage of 500 mg. as the regular vitamin C caplet. The chewable vitamin showed that 34 drops of iodine were needed at the halfway, while 31 drops were needed for the soybeans at this point. At the full time test, the soybeans yielded 52 drops of iodine, while the chewable vitamin C yielded 59 drops. It is obvious that these levels of vitamin C are comparable ad relatively close with the solution containing the soybeans and the solutions containing the vitamins.

The vitamin B-12 test concluded that vitamins are fast-acting or slower/timed-release based on their coating on the outside of the vitamin. Having a capsule or a caplet on the outside of the vitamin prevents the vitamin from immediately dissolving in the stomach acid. As opposed to this, soft gels and chewable vitamins are immediately dissolved into the stomach acid and disperse a lot of vitamin C to be absorbed into the bloodstream with intrinsic factor (IF). The fast-acting vitamin used in this experiment actually held to its name and had a higher absorbency of cobalamin at 0.635 than the timed-release vitamin that had a 0.302 absorbency. Timed-release vitamins are designed to disperse a steady amount of a certain vitamin into the blood stream, rather than all of it being absorbed at once.

**Further Research:**

- No refrigeration of digestive substances (May have affected the results of the full time vitamin C test as the vitamin may have degraded while in the fridge for a week)
- There was no halfway test done for vitamin B12, so there is no way of knowing if there would be more cobalamin in beakers B or C after the digestion period has been completed
- The tests done for Vitamin C and B-12 in the solutions containing digestive substances could have been done differently. A list of other tests includes…
  - Creating a 1% vitamin C solution while also making a DCPIP (Dichlorophenolindophenol) solution that helps test for ascorbic acid content by having a redox reaction occur that turns the solution blue once it reacts with the ascorbic acid
  - Looking at human blood for vitamin B12 deficiency levels as vitamin B12 is absorbed directly into the bloodstream after in binds to intrinsic factor
- Increasing concentrations of HCl and pepsin in the stomach acid solutions to compare the different effects of digestion on the substances
Works Cited


2. Culp, Mary. "How to Construct an Artificial Stomach." The American Biology Teacher  

   12 June 2014.

