Degradation of Vitamin C in Orange Juice Stored at Various Temperatures

Syndy Rielle Malit
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Abstract:
Citrus fruits are well known for their vitamin C content. This study is focused on the degradation of the vitamin C based on temperature storage. By focusing the study on batches of orange juice and not the oranges themselves, the factors of size, shape, and color can be eliminated making the vitamin C content the only variable. The best storage temperature for orange juice, so as to slow down the degrading of the vitamin C, is found to be at lower temperatures. The vitamin C content is analyzed through the use of iodine titration and a starch indicator. An unexpected apparent increase in vitamin C content after an initial decrease was uncovered.

Introduction:
Given the task to find a way to fight off scurvy while on a new reality TV show, “Walk the Plank,” oranges were studied for their vitamin C (Ascorbic Acid, AA) content. The AA found in these citrus fruits should be enough to counter scurvy, but for how long? A minimum of 10mg of AA is necessary to combat the disease.

The loss of AA content in the juice was determined using iodine titrations. Data points were obtained for juices stored at four different temperatures, over a nine-day period.

Method:
A bag of oranges picked from Jacinto Farms in Mentone California were juiced and combined to make a single batch of 400mL. After cutting off the peels from the oranges, the interior portions were then placed into a juicer, the juice was then filtered through cheesecloth to separate the juice from any pulp. Portions of that stock were then stored at temperatures 4.3 °C (Cold Room), 5 °C (Dr. Mink’s Refrigerator), 8 °C (Stock Room Refrigerator), and 24 °C (Room Temperature). A titration of the original juice was done on the first day to find the initial Vitamin C (Ascorbic Acid, AA) content. First a standard of iodine solution was created by titrating it against an AA solution made by dissolving a known amount of AA in 100 mL. Then a 1% starch solution was added to the AA solution as an indicator to signal the end of the reaction by turning the solution blue. After standardizing the iodine solution, a 10 mL juice sample was titrated, once the blue color was reached, the volume of iodine was noted and then converted over to mg/100 mL of AA found in the juice.

The following days, a portion each juice sample stored at different temperatures was similarly titrated to determine the AA content. The titration consisted of 10 mL of juice with 25 drops of 1% starch solution placed into a 125-mL Erlenmeyer flask, titrated with the iodine solution inside a 50-mL buret. For each juice stored at different temperatures three trials of each juice were conducted. The experiment lasted for nine days, where
titrations were only done for six days (no titrations occurred over the weekend or Friday, Days 3-5 the juices were left in storage).

Day 1 a juice batch was created and then split into two plastic bottles stored in a room temperature setting (24°C) and 8°C. Day 2 a second juice batch was created and also split into two plastic bottles and stored in a 4.3°C and 5°C setting, while still titrating the two previous juices stored. Day 6-9 consisted of titrating all 4 of the different juices stored in different temperature conditions.

Data:

### Standardization of Iodine Solution Measurements

<table>
<thead>
<tr>
<th>Day</th>
<th>g of AA</th>
<th>mL AA diluted to</th>
<th>AA concentration (g/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>0.0501</td>
<td>100</td>
<td>0.000501</td>
</tr>
<tr>
<td>Day 2</td>
<td>0.0501</td>
<td>100</td>
<td>0.000501</td>
</tr>
<tr>
<td>Day 6</td>
<td>0.0500</td>
<td>100</td>
<td>0.000500</td>
</tr>
<tr>
<td>Day 7</td>
<td>0.0500</td>
<td>100</td>
<td>0.000500</td>
</tr>
<tr>
<td>Day 8</td>
<td>0.0502</td>
<td>100</td>
<td>0.000502</td>
</tr>
<tr>
<td>Day 9</td>
<td>0.0500</td>
<td>100</td>
<td>0.000500</td>
</tr>
</tbody>
</table>

### Average mL of Iodine Solution Used in Titrations

<table>
<thead>
<tr>
<th>Room Temp (24°C)</th>
<th>Stock Room (8°C)</th>
<th>Freezer (4.3°C)</th>
<th>Dr Mink Fridge (5°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>7.67</td>
<td>7.67</td>
<td></td>
</tr>
<tr>
<td>Day 2</td>
<td>7.42</td>
<td>7.50</td>
<td>7.30</td>
</tr>
<tr>
<td>Day 6</td>
<td>7.25</td>
<td>7.18</td>
<td>7.10</td>
</tr>
<tr>
<td>Day 7</td>
<td>7.25</td>
<td>7.05</td>
<td>7.00</td>
</tr>
<tr>
<td>Day 8</td>
<td>7.60</td>
<td>7.13</td>
<td>7.04</td>
</tr>
<tr>
<td>Day 9</td>
<td>7.53</td>
<td>7.12</td>
<td>7.12</td>
</tr>
</tbody>
</table>

Calculation of titration:

\[
\frac{\text{g of AA used}}{\text{mL H}_2\text{O AA dissolved in}} \times \frac{\text{mL of AA used in titration}}{\text{g of AA used in standardization}} = \text{AA content in 10mL juice sample}
\]

Ex. Day 6 of Stock Room Juice

\[
\left(\frac{0.0500 \text{ g AA}}{100 \text{ mL}}\right)(25.00 \text{ mL}) = 0.0125 \text{ g AA used in standardization}
\]

\[
\left(\frac{0.0125 \text{ g AA}}{18.83 \text{ mL}}\right) \times 7.18 \text{ mL} = 0.004768 \text{ g AA}
\]
**Results:**
With the pre-squeezed orange juice, the AA content showed an initial decline, followed by an increase at Day 6 then finished by lowering of the AA content again. The Room Temperature juice shows the greatest increase of apparent AA content showing a side reaction. The side reaction occurring causes interference with the AA titration and indicated that AA is not the only substance reacting with the iodine titrant.

The other three juice portions show a “leveling out” towards Days 6-9, where their AA stays in the range of 46-48 mg AA/100 mL juice, which could indicate that AA loss has stabilized, or that the secondary substance is being formed at a rate similar to AA loss.

**Discussion:**
Although there shows an increase of the apparent AA content at Day 6, if only the data points that are before the onset of increasing data are used, least-squares analysis can predict an equation for the loss of AA over time.
These equations can then allow us to predict the day on which the orange juice would lose all of their AA contents.

For example taking the equation given from the Room Temperature juice we have:

\[ y = -2.84x + 57 \]

To find the day at which the AA would be 0, set \( y = 0 \)

\[ 0 = -2.84x + 57 \]

\[ 2.84x = 57 \]

\[ x = 20.07 \]

Doing these steps for the other 4 reactions we find that it would take 20 days for all the AA content in juice at Room Temperature to be nonexistent, 23 for the Stock Room, 25 for the Cold Room, and 12 days for Dr. Mink’s Refrigerator. Because only 2 data points were used for Dr. Mink’s linear regression line, the accuracy of that equation in relation to the other equations is not very high.

Evidence for a side reaction occurring could be by observing physical changes in the juice. The juice darkened and the odor changed. In the case for the Room Temperature juice, after leaving the juice over the weekend in storage a rotting banana smell was observed with an alcoholic after smell.

A side experiment was done to see if ethanol was being formed and if that formation would react with the iodine solution. A distillation was performed on 5 mL of the Room Temperature juice. The liquid collected was then examined using the Refractometer and
found that 1% mol fraction of the collected liquid was ethanol. A 1% mol fraction ethanol solution was then created and titrated, and found that the formation of ethanol was not enough to disrupt the titration.

Other factors that may be affecting the titration is human error. A different method to finding the endpoint may want to be found. A color indicator is a simple method, but with different orange juice colors in the beginning, the blue color changes from sample to sample, which will affect the amount of iodine solution being noted.

**Conclusion:**
As the juice ages the study shows a decrease of the AA content, with a side reaction causing an apparent AA content increase. The secondary reaction is occurring during the storage of the orange juice, which interfered with the iodine titration and is most pronounced for the juice stored at room temperature. Further studies should be conducted to find the source of the other substance reacting with the iodine. Going back to the question asked at the beginning of the research period, only 10mg of AA is needed per day to fight off the effects of scurvy. Using the equations found using the Least-Squares Line we set y=10 instead of 0 to find out when the juice will have 10mg of AA content. Room Temperature shows that at 16 days the AA would be 10mg, Stock Room has 18 days, the Cold Room has 20 days, and Dr. Mink’s Refrigerator has 9 days. It seems the best storage temperature is the Cold Room, which shows the AA content in the orange juice lasting up to 20 days. Since the trip would last for 2 months (60 days), a different storage method may want to be examined for the rest of the trip.

**References:**

Dr. Kimberley R. Cousins. “How much C at Sea? A guided case study”


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