Independent Research Investigation

Research Question
Analyzing the effect of cooking time on chlorophyll degradation in broccoli with a spectrophotometer.

Introduction
The appearance of food has always been a crucial factor in our daily consumption decisions. The color plays an important role in our judgment and desirability for certain food. Food industries and restaurants strive to serve products that look appealing to achieve maximum consumption. For example, one is more likely to purchase green, fresh spinach than brown, wrinkled ones. This is arguably why we have spent so much investment to produce food that is not only taste-wise appetizing but also appearance-wise alluring. While food industries invest in food packaging and supermarkets in raw food appearance, at home the focus is on the timing and method of cooking so that food is served with optimum taste and — more importantly — pleasing visual effect. The focus of this experiment is to analyze the effect of heat on chlorophyll degradation in broccoli by using the spectrophotometer. By measuring the effect of cooking time on chlorophyll degradation, the experiment will provide evidence that foods containing chlorophyll will change color during cooking. This will enable all who enjoys food to produce meals with visual appeal.

Background Information

<table>
<thead>
<tr>
<th>Cooking time (minutes)</th>
<th>Percentage Chlorophyll retention</th>
<th>Appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>100</td>
<td>Green</td>
</tr>
<tr>
<td>5</td>
<td>74</td>
<td>Green</td>
</tr>
<tr>
<td>10</td>
<td>62</td>
<td>Green</td>
</tr>
<tr>
<td>20</td>
<td>36</td>
<td>Brownish green</td>
</tr>
<tr>
<td>60</td>
<td>0</td>
<td>Yellowish</td>
</tr>
</tbody>
</table>

Figure 1 Effect of cooking time on chlorophyll retention (Srilakshmi, 2003)
Chlorophylls are green, water-insoluble pigments composed of a porphyrin structure with a central magnesium ion (Derry et al., 2009 p355). However, they are thermally unstable. When heated, the plant cell’s compartmentalization fractures, releasing organic acid and hence lowering the pH. This causes the magnesium in the chlorophyll to be replaced by two hydrogen ions, as seen in figure 2, and to form an olive-green pheophytin complex (Derry et al., 2009 p355).

The pH influences chlorophyll’s thermal stability (Brown & Ford, 2009 p763). As the cooking time increases, the amount of acids released also increases, resulting in more of chlorophyll’s magnesium being replaced, which causes greater chlorophyll degradation. Figure 1 above shows that vegetable’s color changes from green to brownish green, and then to yellowish over a cooking period of 60 minutes.

The spectrophotometer can be used to determine the chlorophyll color. It is composed of two parts: a spectrometer that produces light of specific frequencies and a photometer that measures the intensity of light. Different substances absorb and transmit different wavelengths of light, and the extent of absorption of light of a specific wavelength can determine the concentration of substances in solution (Caprette, 2012).

**Prediction**

If the length of cooking time increases, then the maximum wavelength of absorbance will increase because cooking causes acids to be released within plant cells, causing chlorophyll degradation. As a result, the color observed will change from green to olive green.
Method

Safety Precautions

There aren’t any safety precautions or ethical considerations in this lab.

Procedure

1. Turn on the hot plate.
2. Label five 250mL beakers 1 minute, 5 minutes, 10 minutes, 15 minutes, and 20 minutes.²
3. Using a 50 (±0.5mL) measuring cylinder, measure and pour 30mL of water into each of the five beakers.
4. Place all five beakers on the hot plate at 250°C; temperature of the cooking water needs to be kept consistent, as it will affect the rate of degradation in the rate of acids released.
5. Using an electronic balance (±0.001g), cut and measure five separate 10g samples of broccoli florets from a single broccoli head to minimize differences in broccoli age, color and condition; the broccoli florets should be trialed on the same day to ensure equal freshness and hence colors; the florets’ masses are consistent to produce comparable results.
6. Place a quantitative filter paper on each of the five funnels, and place the funnels into five 50mL conical flasks.
7. When all the cooking water is between 90 and 100°C, put a 10g sample of broccoli florets into each of the five beakers, and start the stopwatch.
8. When the stopwatch reads 1 minute, take the beaker labeled 1 minute off the heat pad with a glove to prevent burning, and pour the cooking water through the funnel and into the flask; it is important that this step happens quickly because broccoli’s presence in the water causes chlorophylls to continue to degrade.
9. Repeat step 8 after 2 minutes, 3 minutes, 4 minutes, 5 minutes, and 10 minutes.
10. Calibrate the spectrometer.²
11. Measure 3mL of each of the filtered cooking water samples and measure the maximum wavelength of light absorbed using the spectrometer. Record the color of cooking water.
12. Repeat steps 2-11 one more time for trial 2.

¹ The time intervals have been modified; the original 1, 5, 10, 15, 20, and 60-minute intervals were changed to 1, 2, 3, 4, 5, and 10-minute intervals because the original time frames are too long and did not reflect the real time for cooking broccoli.
² A colorimeter was originally used, but because it only measures four wavelengths, it is changed to a spectrometer.
Data Collection and Processing

Table 1 Effect of boiling time on chlorophyll degradation

<table>
<thead>
<tr>
<th>Time of Boiling (min) (±10 sec)</th>
<th>Maximum Absorbance Wavelength (±1 nm)</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial 1</td>
<td>Trial 2</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>380</td>
<td>380</td>
</tr>
<tr>
<td>2</td>
<td>380</td>
<td>380</td>
</tr>
<tr>
<td>3</td>
<td>380</td>
<td>380</td>
</tr>
<tr>
<td>4</td>
<td>387</td>
<td>383</td>
</tr>
<tr>
<td>5</td>
<td>386</td>
<td>390</td>
</tr>
<tr>
<td>10</td>
<td>386</td>
<td>390</td>
</tr>
</tbody>
</table>

Average = (T1 + T2)/2
Ex: (386±1 nm + 390±1 nm)/2 = 388±1 nm

Figure 3 Effect of boiling time on chlorophyll degradation

A: Would have been better to record intensity of several major peaks. This data lacks discrimination.

A: This is not really a valid graph. There is no reason to assume that there will be a gradual shift in peak wavelength. It should have been peak intensities that were plotted against boiling time.

3 Estimated time it takes to take the beaker off the hot plate and to filter broccoli florets out from the cooking water.
Investigation 10 (annotated)

Figure 4 A portion of the absorbance spectrum graph for the 5-minute sample in trial 2.

Table 2 Color of water samples cooked at different time intervals

<table>
<thead>
<tr>
<th>Time of Boiling (min) (±10sec)</th>
<th>Color description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>transparent light green</td>
</tr>
<tr>
<td>2</td>
<td>light yellowish green</td>
</tr>
<tr>
<td>3</td>
<td>light yellowish green</td>
</tr>
<tr>
<td>4</td>
<td>medium yellowish green</td>
</tr>
<tr>
<td>5</td>
<td>darker yellowish green</td>
</tr>
<tr>
<td>10</td>
<td>light brownish green</td>
</tr>
</tbody>
</table>

Conclusion
This research investigated the effect of cooking time length on chlorophyll degradation in broccoli. By measuring cooking waters’ maximum absorbance with a spectrophotometer, the colors of the water samples are compared to determine color changes as a result of chlorophyll degradation. Although there is an increase in the maximum absorbance (Table 1), there is a weak linear correlation between cooking time lengths and the...
maximum absorbance. The low R²-value of 0.68 shows that the increase in maximum absorbance is not directly related to the increase in cooking time. Therefore, the initial prediction is not validated in this experiment.

Furthermore, the lack of controlled variables contributes to the experiment’s overall unreliability. Firstly, chlorophyll is highly unstable, and factors such as air and light can also influence its stability (Derry et al., 2009 p355). However, these were not controlled in the experiment. Secondly, pigments aside from chlorophylls, such as carotenoid, also influence the color of the cooking water samples, and hence their maximum absorbance. Thirdly, the data collection is based upon six time intervals with only two trials each, which would cause a higher frequency for errors. Lastly, although the cooking water temperature was supposed to be a controlled variable, it was not controlled, ranging from 90°C to 100°C. Therefore, a conclusion cannot be drawn by the experiment due to the lack of controlled variables in broccoli preparation condition, pigment variations in broccoli, lack of data points, and temperature.

However, the purpose of this investigation is important in that it helps suggest ways to preserve food so that they are fresh and colored when served. Food appearance, and hence their color, makes a tremendous first impression, which is why the method of food preparation, such as cooking, should be controlled. Naturally colored food, such as vegetables, are especially susceptible to color change when cooked, which makes investigation into ways to prevent chlorophyll degradation valuable.

Besides investigating time control in cooking, a further investigation can study how different light and pH environment would effect chlorophyll degradation.

Evaluation

In determining the reliability of the absorbance wavelengths collected for the cooking water samples, one must evaluate the procedure and the method of selecting data for analysis. Although the mass of broccoli florets and volume of water used were kept constant, the cooking water temperature of each individual beaker varied greatly, ranging from 90°C to 100°C. A false assumption was made that all cooking water will have the same temperature as they were all were placed on the same hot plate. However, this was not true; the cooking water temperatures varied with the beaker’s location on the hot plate, as well as fluctuation in the room temperature. The variation in water temperature affects the rate of chlorophyll degradation, because the higher the temperature, the more acids are released, which causes more magnesium to be
replaced by hydrogen ions. Also, a lack of knowledge in water’s boiling point also contributed to the experiment’s systematic errors. Since water boils at a constant temperature, temperature will be maintained and controlled if water boils. Therefore, an improvement to control the cooking water temperature is to place the broccoli florets into the cooking water only when the water is boiling.

The fact that the broccoli head used was not prepared in a controlled environment is also another systematic error. Chlorophyll’s stability is greatly influenced by air and light. However, the broccoli was not sealed, nor was it kept in a dark environment. This might cause the broccoli to be degraded before the experimentation, which would lower the overall difference in maximum absorbance between the different cooking water samples. Therefore, next time, the broccoli should be placed in an airtight zip-lock bag, and stored away from light.

Additionally, the lack of consideration for other pigments within the broccoli also causes systematic error in the maximum absorbance of the cooking water samples. Carotenoid, another pigment found in broccolis, has a color range of yellow to red (Derry et. al, 2009 p354). Its presence would increase the overall absorbance of the cooking water. Therefore, the cooking water’s yellowish color might also be attributed to carotenoids and not solely to the degraded chlorophyll.

Also, the method in choosing the maximum absorbance values used for analysis is a source of random error in the experiment. For some water samples, the graphs fluctuated greatly during data collection. As seen in figure 3, some graphs even have several peak absorbance wavelengths. The wavelengths used for analysis are the highest peaks in each individual graphs, where this method excludes the other peak absorbance wavelengths. The fact that the excluded wavelengths are not included for analysis makes the experiment unreliable. The presence of several peaks within a graph may be due to very fine solids in the solution that obstructs the spectrophotometer’s reading. Therefore, in order to attain an obvious maximum peak, even finer filter papers should be used.

Most importantly, the use of only six time intervals for data collection is the main source of random error. More data points will allow for a more accurate trend line and conclusion. The time intervals should be expanded from six to at least ten, with 1-minute intervals, or even 30-second intervals, within a 1-minute to 10-minute range. Also, the minimal trials conducted also contribute to random errors, since a mistake in a trial will greatly affect the averaged results. Therefore, at least three trials should be conducted.
for each time interval to minimize such mistakes and errors in the final calculation and analysis. With an increase in time intervals and trials, the averaged result will become more reliable and help strengthen a possible linear relationship between cooking time and maximum absorbance.

Word Count: 1742

**Bibliography**


