Raw vs. Boiled Vegetables: Examining Water-soluble Vitamin Content by TLC

Abstract

Vegetables are valuable sources of vitamins your body needs to function. How does the vitamin content of the veggie change depending on how you cook it? Is raw better than boiled? What vitamins are in them? In this experiment, students will investigate the vitamin content of green bell peppers. Appropriate for high school students and adaptable for students of younger grades, this experiment introduces students to the basic separation technique of thin-layer chromatography (TLC), often used in organic labs. A thermal cycler is included to varying temperatures incrementally. Note: Thermal cyclers are essential to the PCR process. This piece of equipment could also be repurposed to accommodate a PCR experiment.

Instruments being donated:

- Thermal Cycler

Goal: Observe what happens to vitamin content upon boiling vegetables using TLC. You will observe how vitamin content changes over time and what vitamins are in solution.

Introduction:

Polarity is a term used to describe how electrons are shared among elements in a molecule. A polar molecule will tend to interact more with other polar molecules. Likewise, nonpolar molecules like to linger with nonpolar molecules and away from polar ones. Thin-layer chromatography (TLC) is a common technique used to separate compounds in a liquid mixture based on polarity. TLC plates used in this experiment are covered in silica, a polar molecule, and developed with a primarily nonpolar solvent. The samples spotted will travel up the TLC plate with the solvent. However, as the compounds move up the plate, the more polar compounds will interact with the polar silica on the plate. Thus, they will move slower than the nonpolar or less polar compounds that are in solution, interact less with polar silica, and end up traveling further up the plate.

Vitamins can be divided into two groups: water-soluble and fat- soluble. Fat-soluble vitamins like vitamin A, D, E, and K are hydrophobic. They do not dissolve in water and are more difficult to extract. However, vitamin C and B-group vitamins are water-soluble. What vitamins are found in green peppers? Since we are testing for water-soluble vitamins, what vitamins do you predict will show up on the TLC analysis?

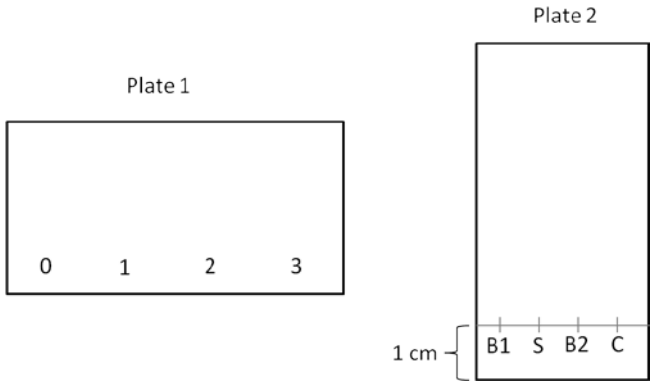
Materials:

- Thermal cycler
- 0.5 ml centrifuge tubes (4 per group)
- Green pepper slice

- Knives
- TLC chambers (jars with caps, 1 per group)
- 80:20 methanol: water solution
- Vitamin standards in methanol
    - B1—Thiamine
    - B2—riboflavin
    - C—ascorbic acid
- TLC plates (2 per group)
- Plastic fine-tipped pipets
- UV light
- Pencils and rulers
- Coffee filters, cut in half
- Gloves

Procedure:

1. Fill a TLC chamber up to 1 cm with the methanol-water solution. Place half of a coffee filter along the sides of the jar. Close the cap and invert the jar to fully soak the filter in the solution. It is important to keep the cap on the jar as much as possible so as to maintain a saturated environment in the jar.
2. Wash the green pepper slices with distilled water. Once the pepper is washed, soak it in distilled water for a minute. Then take out enough water to fill a 0.5 ml centrifuge tube. Label this with a “0”. This sample will represent the vitamin content of raw vegetables initially, before boiling.
3. Finely chop the green bell peppers and fill a 0.5 ml centrifuge tube half-full with the peppers. Add enough water to fill up 2/3 of the tube.
4. Once all the tubes are in the thermal cycler, run the VEGGIE cycle. The cycler will “cook” the vegetables at 100 degrees Celsius for 5 minutes three times. The cycler will cool down for 15 minutes between each round to allow students to retrieve their samples for analysis.
5. After each cycle, take out a small amount of the water (enough to fill 1/3 or less of the tube) and put it into a 0.5 ml centrifuge tube. Make sure you label each tube with the appropriate number of the cycle. Do this three times.
6. While the cycler runs, prepare 2 TLC plates as shown. Draw carefully on the plate with a pencil (must be a pencil!); try not to scratch off the white silica. Make sure the dashes are not too close to the side of the sample otherwise the spots might run off the plate. Avoid contaminating the TLC plate by holding the plate on the edges as much as possible.



7. Spot the plates as shown by the instructor.
    a. Plate 1 monitors the presence of vitamins after each boiling cycle. We are measuring the presence of vitamins that the vitamins that have come out of pepper and into the water. Spot samples from each cycle above each label.
    b. Plate 2 will observe which vitamins are present. Spot the standard solutions provided on the appropriate dash on the line. After you have spotted plate 1, fill up the rest of sample tube 2 with methanol. Spot this solution on the "S" dash.
    c. Note: Only a little bit of solution is needed on each spot. Make sure the spots do not get too large; we do not want them to run into each other when the plate is developing. A circle around 5 mm in diameter is enough
8. Carefully place the TLC plate in the jar, making sure that the edges do not touch the filter paper and the penciled line does not go underneath the solvent line.
9. Allow the TLC plate to develop. The solvent should move up the plate due to capillary motion. Do not disrupt the jar once the plate is inside.
10. When the solvent front has almost reached 1 cm from the top, remove the plate and mark the solvent front with a pencil. Allow the solvent to dry.
11. Once dry, use a UV lamp to observe spots on each plate. Make notes about the intensity of the spots on plate 1. Circle dark spots on plate 2 with a pencil. Record drawings of these plates.
    a. UV rays can damage eyes. Do not look straight into the lamp.

Data Analysis:

1. Based on your observations of plate 1, what can you say about vitamin content remaining in the vegetable as the peppers get boiled? Recall that the sample on the plate is the amount of vitamin in the *water*. Higher amounts of vitamins in the water indicate less vitamin content in the vegetable. Do raw or boiled vegetables retain more vitamins?
2. On plate 2, measure the distance each spot traveled from the starting line. Measure from the starting line to the center of the spot. Also, measure the distance of the solvent front from the starting line.

3. Calculate the R_f value using the equation:

$$R_f = \frac{distance\ traveled\ by\ spot}{distance\ traveled\ by\ solvent\ front}$$

4. Compare R_f values of your vegetable sample to the vitamin standards to determine which vitamins are present.

Discussion Questions:

1. How does TLC work? Explain in your own words.
2. What is the purpose of calculating an R_f value?
3. What is a polar compound?

For the Teacher

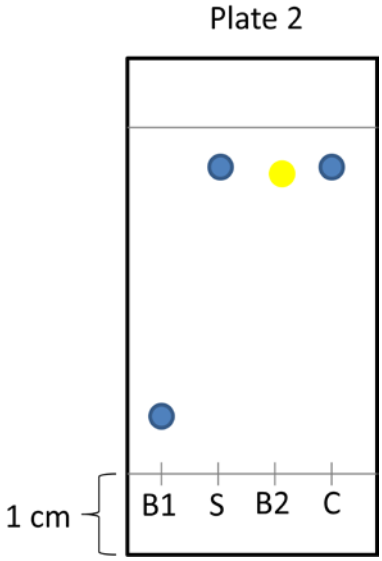
Prep:

1. Dissolve vitamin standards in methanol to a concentration of 1 mg/ml.
2. Slice a green bell pepper into slices.
3. Mix a 80:20 methanol:water solution in a glass capped bottle. (90:10 works well too)
4. TLC plates will need to be cut into smaller pieces, small enough to fit in the TLC chambers. TLC plates should be around 1" X 2.5" Plate can be cut using the typical paper cutting board.
5. Make spotters.

Analysis Notes:

An ideal green pepper plate will look like this:

- B2 will show up as a yellow spot but will show no dark spot under the UV
- B1 and C will only be visible under the UV
- The green pepper sample contains vitamin C because there is no yellow color. There is a dark spot which is indicative of vitamin C.



Clean-up:

1. Peppers and TLC plates can be disposed of in the trash. If glass spotters are used, dispose like broken glass.
2. Do NOT pour methanol down the drain. Collect solvent solutions from students and dispose accordingly. If a hood is available, the methanol can be allowed to evaporate in the hood.

Future Experiments:

- To save time, you can only run two cycles on the cycler.
- Raw vs. Steamed? Mashed up?
- Try other vitamins and other vegetables (though you may have to adjust the solvent ratios to obtain sufficient separation)

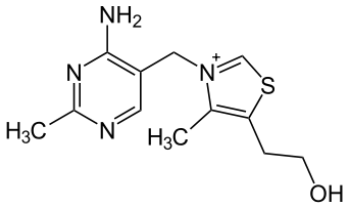
- B3—niacin, nicotinic acid (100g, AAA12683-30)
- B6—pyridoxal phosphate (5 g, 89151-416)

- For more advanced students, you could allow students to formulate their own hypothesis and experiment, designing their own TLC plates to run and which solvent ratios to use.

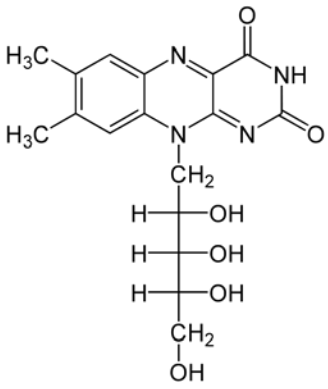
Product Reorder list:

- Centrifuge tubes: 0.5 ml (89000-010)
- Scintillation vials for the standards (20 ml): (66022-274)
- Methanol (BDH1135-1LP)
- Vitamin Standards (or by them from a vitamin store):
  - B1—Thiamine (100 g, AAAA19560-22)
  - B2—riboflavin (25g, AAA11764-14)
  - C—ascorbic acid (25 g, 95031-858)
- TLC plates with fluorescence indicator (20 plates, EMD-5549-4)
- Plastic fine-tipped pipets (Pack of 500, 16001-192)
- UV light: long and short wave (89131-492)
- Spatula (82027-528)

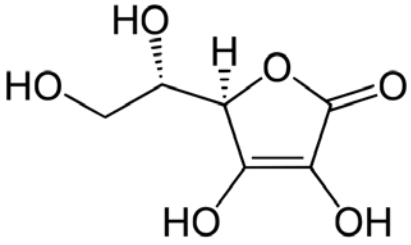
Chemical structures:



Thiamine (B1)



Riboflavin (B2)



Ascorbic Acid (C)