

## Chapter 7

# Measuring the Amount of Ascorbic Acid in Cabbage

*Carol Reiss*

Section of Plant Biology  
Division of Biological Sciences, Cornell University  
Ithaca, New York 14850

Carol Reiss received her B.A. in Russian Literature from Cornell in 1966 and M.S. in Botany from Cornell in 1970. She is currently a Senior Lecturer in the Section of Plant Biology at Cornell and has been coordinating and teaching Plant Physiology laboratory classes at Cornell for 16 years. Her research interests include the involvement of calcium in chlorophyll synthesis and the development of undergraduate teaching laboratories in plant physiology. She is currently writing a laboratory manual in plant physiology to be published by Prentice Hall in 1993. The experiment in this chapter will be included in this manual.

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## Introduction

This experiment has been used as the introductory experiment for the Plant Physiology laboratory courses at Cornell for over 15 years; it has proved to be a valuable starting point for students with a limited background in biology and chemistry.

As stated in the Introduction for the students, this experiment has two goals: to determine (1) the amount of ascorbic acid present in fresh cabbage tissue and (2) if boiling the cabbage in water for 5 minutes destroys the ascorbic acid present in the fresh tissue. In addition, this exercise introduces the student to the problems involved in the design and interpretation of simple experiments and emphasizes that experiments with biological systems require at least as much care and attention to detail as experiments in chemistry or physics. The determination of the ascorbic acid content of cabbage serves to illustrate the general problems involved in quantifying components of the plant cell.

Although students in the Plant Physiology laboratory classes at Cornell have taken prerequisite courses in Introductory Biology and Introductory Chemistry, almost no biology background is necessary to complete this experiment. The instructor's preparation time is less than 2 hours; the student experiment may be completed in 3 hours. Many variations on the basic experiment are possible; for example, a comparison of the outer cabbage leaves with stalk tissue, a comparison of the ascorbic acid content of cabbage with other vegetables or fruits, or a time course for boiling.

## Student Outline

### Introduction

Although you may associate vitamin C with citrus fruits, ascorbic acid is found in a wide variety of plant tissues. Ascorbic acid is an excellent reducing agent and most likely acts in such a capacity in the plant cell. It is commonly associated with chloroplasts and is present in quantity in green leafy tissues, as found in a head of cabbage.

This experiment has two goals: to determine (1) the amount of ascorbic acid present in fresh cabbage tissue and (2) if boiling the cabbage in water for 5 minutes destroys the ascorbic acid present in the fresh tissue. Some hints about extracting the ascorbic acid and a method for determining ascorbic acid content are included in the Procedure section. You are asked to design the actual procedure for this experiment yourself, using the information provided. You will then complete the experiment following your own protocol to achieve the goals stated above.

It is hoped that this exercise will introduce you to the problems involved in the design and interpretation of simple experiments and emphasize that experiments with biological systems

require at least as much care and attention to detail as experiments in chemistry or physics. The determination of the ascorbic acid content of cabbage will serve to illustrate the general problems involved in quantifying components of the plant cell. Read the discussion that follows (including the Calculations section) and write out your protocol in the space provided before starting the experiment.

### Materials

#### *Equipment*

Knives, large  
Balances and weighing paper  
Mortars (400 ml) and pestles  
Sand  
Miracloth  
Funnels, 150 mm  
Graduated cylinders, 250 ml, 500 ml  
Pipets, 10 ml  
Burets, 50 ml  
Heat resistant gloves  
pH meter  
Pasteur pipets and latex bulbs  
Thermometers

#### *Solutions*

Ascorbic acid, 4.0 mg/ml (kept cold and in the dark)  
5% metaphosphoric acid  
Dichlorophenol-indophenol (DCIP), 0.8 g/liter

#### *Plant material*

Green cabbage (*Brassica oleracea*)

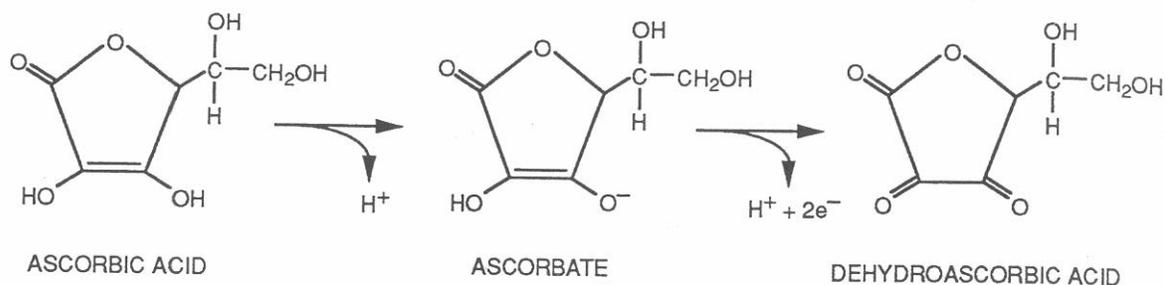
#### *Clean up*

Waste container for DCIP

### Procedure

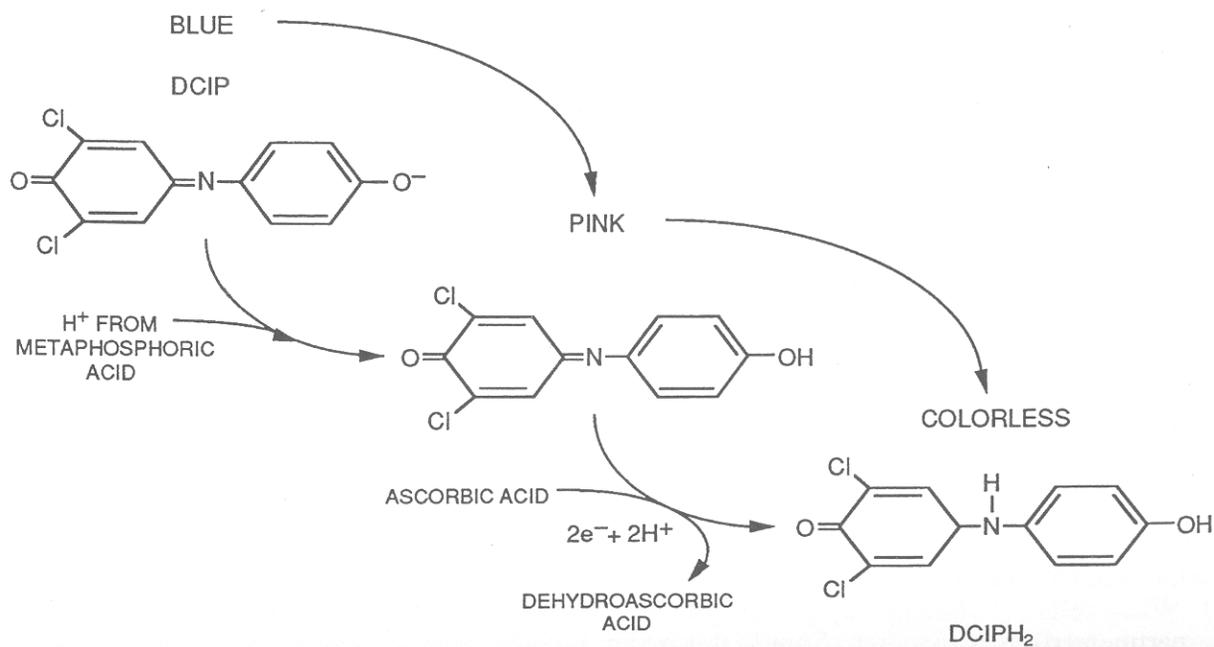
The ascorbic acid must first be extracted from the cells by breaking the tissue in a medium suitable for extraction. Cabbage tissue, like many higher plant tissues, can be readily homogenized by grinding in a mortar and pestle with a little clean sand (to make the process easier).

For an accurate measurement of the ascorbic acid content, the extraction of ascorbic acid must be complete and no ascorbic acid may be lost to degradation. Many plant tissues contain the enzyme ascorbic acid oxidase, which catalyzes the oxidation of ascorbic acid to dehydroascorbic acid (Figure 7.1). When cells are disrupted by grinding, cell components that are usually separated by membranes (compartmented), mix together. Should this occur, ascorbic acid oxidase may catalyze the oxidation of all the ascorbic acid originally present in the tissue. In order to avoid the loss of ascorbic acid, you are advised to grind the tissue in 5% metaphosphoric acid, which will inactivate the oxidase. Be sure that the cabbage is well immersed in the acid before you begin grinding. After the tissue is thoroughly ground, the homogenate may be filtered by passing it through Miracloth.



**Figure 7.1.** Ascorbic acid and its oxidation to ascorbate and dehydroascorbic acid

The hydrogen atoms of the two enol groups of ascorbic acid may be readily oxidized (Figure 7.1), making ascorbic acid a strong reducing agent. We can take advantage of this property to measure the amount of ascorbic acid present in cabbage. The dye 2,6 dichlorophenol-indophenol (DCIP) is blue in alkali, pink in acid, and can be reduced by ascorbic acid to a colorless “leuco” form (Figure 7.2). If a drop of the blue dye is added to an acidified extract, it will turn pink, then colorless.



**Figure 7.2.** The reduction of DCIP to DCIPH<sub>2</sub>.

When all of the ascorbic acid in the extract has been converted to dehydroascorbic acid, no more  $e^-$  will be available to reduce a drop of DCIP to the colorless form and the solution will remain pink. Therefore, assuming that ascorbic acid is the only substance present in the cabbage extract that will reduce the dye over the range of pH 1 to 4, the amount of ascorbic acid in an extract can be measured by titration against a dilute solution of dye.

The DCIP solution must first be standardized against a known amount of ascorbic acid. This may be accomplished by titrating the dye into a solution containing 1.0 ml of ascorbic acid solution (4.0 mg/ml) and 9 ml of 5% metaphosphoric acid. The end point of the titration will be defined as a pink color that persists through at least 15 seconds of swirling. The amount of ascorbic acid equivalent to 1.0 ml of dye is then calculated.

Carry out the titrations of your extracts as for the standard and follow the instructions in the Calculations section to determine the ascorbic acid content of fresh tissue and tissue that has been boiled for 5 minutes. Record your data in the space provided. The reported values for the ascorbic acid content of cabbage vary over the range of 20–60 mg/100 g fresh weight.

### Calculations

*To standardize the dye:*

Divide 4.0 mg (the amount of ascorbic acid present in the standard solution) by the number of ml of dye titrated to determine the amount of ascorbic acid equivalent to 1.0 ml of dye:

$$\frac{\text{ascorbic acid (mg)}}{1.0 \text{ ml of dye}} = \frac{4.0 \text{ mg of ascorbic acid}}{\text{dye titrated (ml)}}$$

*To determine the amount of ascorbic acid in an aliquot of extract:*

$$\text{mg of ascorbic acid per aliquot} = \text{amount of dye titrated (ml)} \times \frac{\text{ascorbic acid (mg)}}{1 \text{ ml of dye}}$$

*To determine the amount of ascorbic acid in 100 g of cabbage:*

$$\frac{\text{ascorbic acid (mg)}}{100 \text{ g of cabbage tissue}} = \text{mg of ascorbic acid per aliquot} \times \frac{\text{total volume of extract (ml)}}{\text{volume of aliquot (ml)}} \times \frac{100}{\text{weight of cabbage (g)}}$$

Determine the amount of ascorbic acid present in your extracts and record the amounts on the chart provided. Be sure to consider the number of significant figures that are appropriate in your final answer.

**Report**

Name:

Date:

1. Write out your protocol. List **all** of the methods used.

*Results:* Record your data in the spaces provided.

2. To standardize the dye (using 1.0 ml of the ascorbic acid solution and 9 ml of the metaphosphoric acid):

Trial #	Initial buret reading	Final buret reading	Dye titrated (ml)
Dye titrated (average ml):			

Amount of ascorbic acid equivalent to 1 ml of dye: \_\_\_\_\_

3. Fresh tissue:

Weight: \_\_\_\_\_ Total volume: \_\_\_\_\_ Aliquot volume: \_\_\_\_\_

Aliquot #	Initial buret reading	Final buret reading	Dye titrated (ml)
Dye titrated (average ml):			

(a) Determine the average ascorbic acid content (in mg) in an aliquot:

(b) Calculate the ascorbic acid (mg)/100 g cabbage tissue for fresh tissue:

## 3. Boiled tissue:

Weight: \_\_\_\_\_ Total volume: \_\_\_\_\_ Aliquot volume: \_\_\_\_\_

Aliquot #	Initial buret reading	Final buret reading	Dye titrated (ml)
Dye titrated (average ml):			

(a) Determine the average ascorbic acid content (in mg) in an aliquot:

(b) Calculate the ascorbic acid (mg)/100 g cabbage tissue for boiled tissue:

## 4. Boiled water:

Weight: \_\_\_\_\_ Total volume: \_\_\_\_\_ Aliquot volume: \_\_\_\_\_

Aliquot #	Initial buret reading	Final buret reading	Dye titrated (ml)
Dye titrated (average ml):			

(a) Determine the average ascorbic acid content (in mg) in an aliquot:

(b) Calculate the ascorbic acid (mg)/100 g cabbage tissue for the boiled water:

*Summary*

- Record the amount of ascorbic acid (mg/100 g cabbage tissue) for fresh and boiled tissue. Be sure to include the amount of ascorbic acid found in the water in your calculations for boiled tissue.

Amount of ascorbic acid (mg/100 g fresh weight)	
Fresh tissue	
Boiled tissue	
Boiled water	
Total ascorbic acid extracted from boiled tissue	

**Questions**

- Bearing in mind that a whole cabbage is too cumbersome to grind, what is the ideal tissue sample of cabbage for this experiment? Why? Consider what plant parts a cabbage head contains. Use a drawing to help explain your answer.
- What is an aliquot and how is it useful?
- If ascorbic acid was lost from the tissue during the boiling procedure, what happened to it? What evidence from your experiment supports this conclusion?
- Explain how your choice of boiling procedure affected the total amount of ascorbic acid extracted.
- If you were to repeat this experiment, how would you change it to reduce the loss of ascorbic acid during extraction? Be specific.

## APPENDIX A

### *Preparations*

#### **Solutions**

The amounts given will be sufficient for a class of 12 students, working in groups of two.

1. 4000 ml of 5% metaphosphoric acid [200 g/4000 ml]
2. 1000 ml of dichlorophenol-indophenol (DCIP) [0.8 g/1000 ml]
3. 50 ml of ascorbic acid (4.0 mg/1.0 ml) [0.20 g/50 ml]; cover with aluminum foil and refrigerate.

#### **Plant material**

Green cabbage may be purchased at the supermarket. One head is needed per class. Virtually any green vegetable or citrus fruit may be used instead.

#### **Alternatives**

Use other fruits or vegetables and compare to cabbage. Compare ascorbic acid content of leaves to that of the stem. Boil cabbage for different lengths of time.

**Class time:** 3 to 3.5 hours

APPENDIX B  
*Ideal Protocol*

**Fresh Cabbage**

1. Cut a wedge of cabbage and weigh it. It should be between 80 g and 100 g.
2. Quickly chop the cabbage and place it in a mortar with a little sand and cover it with 5% metaphosphoric acid. It is helpful to cut the cabbage further with scissors while it is submerged in the acid. Mash the cabbage with a pestle until a slurry is formed. Filter the mash through Miracloth. Make sure to squeeze the Miracloth to get all the liquid and wash the mash with additional metaphosphoric acid.
3. Measure the total volume.
4. Take 10 ml aliquots and titrate several times with DCIP.

**Boiled Cabbage**

1. Cut a wedge of cabbage and weigh it.
2. Boil the wedge and note boiling procedure (i.e., started in cold or already boiling water). Separate the tissue from the water by filtering through Miracloth.
3. Prepare the boiled tissue extract as for fresh tissue (as above).
4. Measure the pH of the water and acidify it to below pH 4.
5. Measure the total volume of the water.
6. Titrate aliquots of the tissue extract and the boiled water with DCIP.

APPENDIX C  
*Results*

The reported range for the ascorbic acid content of cabbage is 20–60 mg/100 g fresh weight. The following results were obtained from a student laboratory:

Ascorbic acid content (mg/100 g fresh weight) of cabbage tissue				
Started in	Fresh	Boiled	Water	Total
Boiling water	22	10	19	29
	29	19	13	32
	27	8.3	27	35
	25	7.8	28	36
Average	26			33
Cold water	31	7.2	19	26
	30	7.6	17	25
	29	17	7.5	25
	27	13	5.3	18
Average	29			23

The values for fresh tissue fell within the range of reported values for ascorbic acid (mg/100 g fresh weight), although at the lower end of the expected range. Ascorbic acid was most likely lost during extraction for points 1 and 3 given in Appendix D (Sources of Error). Much of the ascorbic acid moved into the water during boiling. The total amount extracted from tissue placed in already boiling water was greater than that extracted from fresh tissue. Boiled tissue was easier to grind, so the extraction process was more efficient. When the tissue was placed into cold water and the temperature was slowly raised, the total amount extracted was less than extracted from fresh tissue. As the temperature rises, membranes will break, allowing ascorbic acid to leak into the water. Ascorbic acid oxidase may be released as well; the oxidase would continue to work on the ascorbic acid until the enzyme is denatured by the higher temperature. When the tissue is placed into already boiling water, the membranes will rupture, but the enzyme will be denatured immediately, preventing the oxidation of ascorbic acid present in the water.

APPENDIX D  
*Sources of Error*

The following points are frequently overlooked by students in the determination of the experimental protocol:

1. The most representative sample of the cabbage is one corresponding to a “pie slice.” Any other sample will not contain amounts of stem, inner leaves, and outer leaves proportional to the amounts in the whole cabbage. Since the ascorbic acid content in these tissues may vary, the measurement would not represent the cabbage as a whole.
2. The total volume of the extract must be measured. The measurement must be as accurate as possible; an approximation of volume would greatly increase the error in the final calculation.
3. All of the ascorbic acid may not be recovered. Spills and incomplete grinding lead to some loss. The liquid in the residue collected in the Miracloth will contain some ascorbic acid. Much of it may be recovered by washing with extra metaphosphoric acid.
4. The extract should be split into aliquots before titration. This enables the student to allow for overshooting the end points in the first titration and allows the student to check the results. It also saves time and dye. However, the measurement of aliquot volume must be accurate; the use of pipets is recommended.
5. There are two ways of boiling cabbage: placing it in cold water first or straight into boiling water. A comparison of the two methods may be instructive.
6. Boiling causes the ascorbic acid to leak out into the water. It is, therefore, necessary to titrate the water as well as the cabbage.
7. The boiled water and cabbage extract must be acidified before titration.
8. Determination of the endpoint is subjective and may result in variations in the determined concentrations between groups.
9. When ascorbic acid is dissolved in an aqueous solution it is readily oxidized by the air. The boiling time should be carefully measured and the boiled water should be rapidly cooled and tested for ascorbic acid.