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ABSTRACT

This publication is the fourth of a series of seven supplementary investigative materials for use in secondary science classes providing up-to-date research-related investigations. This unit is structured for grades 9 through 12. It deals with physical factors that affect color changes in plant foods during processing and in the preparation of fresh and frozen green vegetables for consumers. In addition, this guide provides background information on plant pigments and the changes they undergo during food processing. The first part of this guide identifies: (1) materials needed; (2) supplementary information; (3) suggested reading; and (4) films and film loops. The second part provides students with background information and two investigations: (1) observations on color changes in selected plant material, and (2) factors that affect stability of chlorophyll in green vegetables. Each investigation consists of: (1) materials needed for a four-student team; (2) procedures; (3) questions for thought; (4) extending the investigation; and (5) suggested readings. (HM)

science study aids

4

plant pigments: studies in color changes

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TO THE TEACHER

This Science Study Aid (SSA), structured for grades 9 through 12, is based on research conducted by the Agricultural Research Service at Berkeley, California, and at other ARS laboratories throughout the United States. It deals with physical factors that affect color changes in plant foods during processing and in the preparation of fresh and frozen green vegetables for consumers. In addition, this SSA provides background information on plant pigments and the changes they undergo during food processing. Finally, it allows students to perform laboratory investigations on the treatment of fresh vegetable material and on the removal and subsequent study of their pigments.

The activities are arranged in order of increasing difficulty. Most activities can be carried out using standard laboratory equipment or improvised equipment found in the home, supermarket, or hardware store.

Special precautions must be taken when working with the solvents used to extract plant pigments. Never permit open flame or heating elements to be used anywhere in the room during the extraction process and always work in a well-ventilated room. If your room is equipped with a hood, do not neglect to use it. It is strongly recommended that students carry out the extraction process under the constant supervision of the instructor.

This SSA uses the inquiry method to help develop meaningful group or class discussions. Also, for students who show special interest and for the more able learner, extensions of each of the Investigations are provided.

The material will be of particular value in biology courses during studies of plants,

MATERIALS LIST

For your convenience, the materials needed to perform the Investigations in this Science Study Aid are listed below. The list gives the quantities needed for each team of two or more students.

STUDENT MATERIALS

1 apple
1 banana
1 tomato
1 potato
1 lemon
1 orange
fresh broccoli spears
fresh spinach
vinegar
sodium bicarbonate
sand
distilled water
wax paper
Saran film (only)
15 8" dia. culture dishes
1 250 ml beaker
15 scissors
15 marking pencils
6 hot plates
6 800 ml beakers or pyrex sauce pans
6 large forceps or tongs
10 rulers (mm)
toothpicks
labels
1 stapler
10 mortar and pestle sets
1 one quart screw cap jar
20 small glass containers with cork stoppers
chromatography jar or 1 gal.
wide-mouth mayonnaise jar
chromatographic paper or filter paper
460 ml petroleum ether
40 ml acetone

TEACHER MATERIALS (OPTIONAL)

1 household blender

photosynthesis, energy cycles, or nutrition. It also might be introduced as supplementary material in high school chemistry classes during units of instruction in organic chemistry or biochemistry. An essential part of this SSA is the background material. We recommend that students receive the background information a few days prior to starting the laboratory investigations. This will allow sufficient time for students and the teacher to survey the suggested reading material at the back of the SSA, along with teacher-assigned readings from textbooks.

It is strongly recommended that the students work in teams as they carry out the activities in this Science Study Aid.

INVESTIGATION 2: ALTERNATE METHOD FOR CHLOROPHYLL PREPARATION

Instructions have been provided for student extraction of chlorophyll. However if you personally wish to prepare enough chlorophyll extract for the entire class, follow the instructions given below:

1. In a household blender, with a glass container only, blend 50 gram samples of the vegetable with 100 ml of 100% acetone for 2 minutes.
2. Blend the above mixture 2 additional minutes with 50 ml of fresh, pure acetone.
3. Pour the extract through filter paper into a 250 ml beaker or equivalent container.
4. Repeat the procedure using a clean filter paper, then divide the extract among the student teams.

SUGGESTED READING

Food Technology 11, 109, Dietrich, W. C., Lindquist, F. E., Miers, J. C., Bohart, C. S., Neumann, H. J., and Talburt, W. F. 1957. The time-temperature tolerance of frozen foods. IV. Objective tests to measure adverse changes in frozen vegetables. Good material on factors that produce adverse changes in frozen vegetables.

A Source book for the Biological Sciences, 2nd ed., Morhold, E., Brandwein, P. F., and Joseph, A. 1966. Harcourt, Brace & World, New York, Chapter 2. The so-called "bible" of teachers of the biological sciences. Be sure to order the 2nd edition.

Food Research 23, p. 635, Sweeney, J. P. and Martin, M. E. 1958. Determination of chlorophyll and pheophytin in broccoli heated by various procedures. Two excellent papers that will assist teacher and student alike with research procedures and laboratory techniques.

FILMS

Paper Chromatography (sound, color, 12 min.). Purchase or rent (BSCS). Thorne Films. Excellent teaching aid. Emphasizes basic laboratory techniques in paper chromatography.

Principles of Chromatography (sound, color, 21 min.). Purchase or rent. McGraw-Hill. Presents basic principles of chromatography including techniques used under advanced laboratory conditions.

FILM LOOPS

Identifying Liquids by Chromatography (color, 4 min.). Purchase or obtain through local school system. Earling. Excellent self-study guide for students. May be employed in self-directed studies fo the more able learner.

TO THE STUDENT

BACKGROUND

Why do people buy the things they do in the produce section (fresh fruits and vegetables) of supermarkets? Why do they purchase some items and reject others? Can you think of reasons why many people prefer fresh and frozen green vegetables to canned products?

To begin, what we call "quality" in fruits and vegetables depends on a number of physical characteristics. Three of the most obvious and significant are color, flavor, and texture. Of these, color is of great importance in consumer acceptance. Scientists have estimated that "eye appeal" represents approximately 45% of the total quality scale of color, odor, texture, and taste. Thus, we purchase the fruits and vegetables that look good.

During processing and storage, plant materials undergo a number of physiological and chemical changes. One of the most important is the loss of their green color. As you may know from your studies in biology, chlorophyll is the substance which gives plants their characteristic green color. You will recall that chlorophyll is found in all photosynthesizing organisms. During the processing, storage, and cooking of vegetables, chlorophyll is broken down into substances that often result in less desirable color changes, e. g., fresh or frozen peas versus canned peas. Therefore, chlorophyll retention is used as a measure of quality in green vegetables.

Scientists of the Agricultural Research Service (ARS) of the U. S. Department of Agriculture (USDA) study many problems related to color changes in food products during processing. Some of their research revolves around ways of preventing color changes which result from the chemical alteration of plant pigments. This change in the chemical nature of plant pigments, in many cases, is brought about by periods of long storage, temperature changes, freezing, cooking, and by the use of additives or preservatives, to name only a few. Can you think of other physiological factors that might chemically alter plant pigments?

One area of special interest to ARS scientists concerns the factors that affect the stability of chlorophyll in green vegetables. In order to study this problem, the researcher must, of course, have a clear understanding of chlorophyll. In addition, he must become acquainted with the techniques involved in the removal and study of plant pigments. Both are necessary to insure success.

Suppose you are a scientist working on the problem of chlorophyll stability in green plants after harvest. Do you think that reference materials in your room or library might give you a better understanding of the chemical nature of chlorophyll? Chances are, your teacher has assigned readings on the subject from your textbook and you may wish to review this material.

What kinds of information would you attempt to gather and study concerning the chemical makeup of this vitally important plant pigment? As a guide to your review of the literature, consider the following questions:

Is there more than one kind of chlorophyll found in various plant cells?

If there is more than one kind of chlorophyll, is one form more abundant?

Likewise, is one form of chlorophyll more important?

What colors characterize chlorophyll?

What elements are found in a chlorophyll molecule and what is the ratio of one element to another within this compound?

Obviously, there are questions that you can think of that are not listed above. Include any additional questions that will assist you in your library research.

A second task is to familiarize yourself with the techniques used to extract (remove), separate, and identify plant pigments. One extremely valuable technique, widely used by scientists in separating substances, is called chromatography. This method is used to separate complex mixtures into their individual components. Chromatography also prevents, in many cases, the destruction of unstable compounds that occur if some other analytic method, such as crystallization or distillation, is employed. Furthermore, many separations previously impossible to make can be accomplished by this technique. In certain forms, chromatography may offer a simplicity of operation which allows a much more rapid accumulation of data than that offered by other techniques.

In high school laboratories, the two most common kinds of chromatography used are column chromatography and paper chromatography. In column chromatography, separations are achieved by filtration of a solution having various pigments through a column of finely-divided adsorbing material. The pigments are adsorbed on this material and can be removed later by passing certain solvents through the column. Since the different compounds in the pigment being studied are not removed with the same ease, some will move along the column faster than others.

In paper chromatography, sheets or strips of filter paper can serve as the adsorbing material used in the columns. In this procedure, much smaller amounts of the mixtures being tested are required, and it takes much less time to separate the components making up the mixture. The following Investigations use paper chromatography as the technique for your study of plant pigments. However, you may wish to ask your teacher to design an Investigation based on this SSA that will provide your class with the opportunity to do column chromatography.

Like ARS scientists, you will consider the research problem of chlorophyll stability in vegetables. Your literature survey should provide you with helpful information on the chemical nature of chlorophyll and other plant pigments. The Investigations which you are about to do are designed to help you understand the purpose for doing them and the procedure to follow for successful completion of your research. Finally, a successful research program always uncovers new questions which lead to new assumptions along with schemes to test these assumptions. The careful and informed researcher will always generate more questions than his present investigative activities can answer.

How about you? Will your work lead to new and more interesting ideas related to the study of plant pigments? We sincerely hope it will.

INVESTIGATION 1: OBSERVATIONS ON COLOR CHANGES IN SELECT PLANT MATERIAL

As you progress through this Investigation, see if you can predict what will happen -- formulate assumptions -- and then make educated guesses, based on the data you collect. In order to do this, you will be required to keep accurate records of the observations made during the Investigation. What relationship will your data and assumptions have to the questions listed at the end of the following laboratory activities?

MATERIALS (EACH TEAM)

Part A

1 apple
1 banana
1 tomato
1 potato
1 orange
1 lemon

15 8" dia. culture dishes
250 ml beakers
15 scissors
15 marking pencils
wax paper
Saran film (only)

Part B

6 hot plates
6 800 ml beakers or Pyrex sauce pans
10 ml white vinegar or acetic acid
2 grams sodium bicarbonate
6 large forceps or tongs
2 pkgs. fresh or frozen broccoli or spinach
distilled water

PRELIMINARY PROCEDURE

1. Bring to the laboratory the fresh fruits and fresh or frozen vegetables listed under Materials.
2. Arrange all materials in your work area to follow the sequence of activities outlined in the Procedure following.
3. Design data tables in your notebook based on the observations outlined in the Procedure.

PROCEDURE A

1. Place freshly cut thin slices (1/8" x 1/4" x 1/2") of potato, banana, apple, orange, tomato, and lemon in the bottom of an 8" culture dish. Mark one dish "control." Record in your data table the color and appearance of the freshly cut fruits and vegetables. At the end of the period, make a second observation and record any color changes that have occurred. Cover the dish with a piece of waxed paper and set it aside until the following day, when you will make your final observations. When you come into the laboratory on the following day, remove the waxed paper and record your final observations. Record any color changes that have occurred.
2. Prepare a second and third 8" culture dish with additional thin slices of fruits and vegetables. In the second dish, carefully wrap each slice of plant material with a double layer of Saran film. Be sure the plastic film comes in close contact with the surface of each slice. Attempt to exclude all air! Mark this dish "Experimental Test 1" and make the same observations outlined in Step 1. Cover the dish with Saran film and set it aside until the following day.
3. Then prepare slices for the last dish. Take the remaining pieces of lemon and orange and squeeze the juice from each piece into a beaker. Dip each piece of the freshly sliced fruits and vegetables in the juice-filled beaker and place them in "Experimental Test Dish 2". Again make observations and record your data as outlined in Step 2.

PROCEDURE

1. Design a data table to record your observations during this procedure.
2. Fill an 800 ml beaker with 500 ml of distilled water. Bring the water to a boil and add three fresh or frozen broccoli spears. At intervals of 10, 20, and 30 minutes, remove one broccoli spear, using large forceps, and allow the plant material to drain and immediately cool each spear in cold running tap water. After the last broccoli spear has been removed, place all three against a white background (white enameled pans, paper trays, or paper plates work well) and determine any color changes that have taken place. Record your observations in your data table. Do you think it will be necessary to place one additional broccoli spear in cold distilled water for 30 minutes? Why?
3. Repeat the procedure outlined in Step 2 above, only this time add to the boiling

water 10 ml of white vinegar or 10 ml of a 5% solution of acetic acid. Compare the samples cooked for the recommended time intervals and record all observations in your data table.

4. Again, repeat the procedure outlined in Step 2 above, but use 1 gram of sodium bicarbonate (baking soda) in place of the acetic acid or vinegar. Compare the samples cooked for each of the recommended time intervals and record all observations in your data table. Of what value would the broccoli spears cooked in distilled water (Step 2) be in determining color changes that may have occurred in Steps 3 and 4?

5. Review and think about the data you have recorded. Then write up the results of your investigation under Parts A and B by taking into consideration the following questions.

QUESTIONS FOR THOUGHT

1. In Part A of the Investigation, which plant materials showed definite color changes in the control dish? Did color changes take place in Experimental Test Dishes 1 and 2? If so, in which plant materials did the change occur?
2. In what specific way did Test Dishes 2 and 3 differ from the control dish? Can you suggest why Saran film might be called a mechanical barrier? What might it have prevented from reaching the exposed cells on the cut surface of the plant material?
3. Based on your observations of Test Dish 3, can you explain why people add small amounts of lemon juice to fresh sliced peaches or avocado dip?
4. What pigment is responsible for the color of broccoli?
5. What physical factor used in Part B of the Investigation accelerates the degradation and breakdown of chlorophyll? What chemical factor retarded the degradation of chlorophyll? Which one increased the breakdown rate of chlorophyll?
6. How might the data from Part B of this Investigation suggest ways to improve the appearance of processed food for the consumer?

EXTENDING THE INVESTIGATION

1. Design an investigation to test the color changes in green vegetables cooked in buffer solutions with a pH range of pH 3.0 to pH 8.0. Attempt to establish the optimum pH for chlorophyll retention without affecting texture and flavor. Select a panel of students to carry out tests to determine the most appealing color, flavor, and texture and relate your findings to the optimum pH for color retention.
2. Design an investigation to establish the most favorable cooking time for retaining chlorophyll in selected green vegetables. Collect several wild plants and test for the effect of heat and pH on chlorophyll retention. Are some more resistant than others?

SUGGESTED READING

Biochemistry of Foods, Eskin, N. M. A., Henderson, H. M., and Townsen, R. J. 1971, Academic Press, New York, 240 pp. Excellent information on color changes in fruits and vegetables including chapters on the problem of enzymatic and non-enzymatic browning.

Food Technology, Vol. 5, p. 263, Sweeney, J. P., and Martin, M. E. 1961. Stability of chlorophyll in vegetables as affected by pH.

RESEARCH INVESTIGATION 2: FACTORS THAT AFFECT STABILITY OF CHLOROPHYLL IN GREEN VEGETABLES

Purpose

In this investigation you will treat fresh or frozen vegetables by varying their cooking time and pH. Following this variation in treatment, you will use the techniques of paper chromatography in an attempt to make judgments about the effect of various physical and chemical factors on chlorophyll breakdown. Scientists have discovered that one of the breakdown products of chlorophyll is a compound called pheophytin. The formation of this compound is directly responsible for some of the undesirable color changes that take place when green vegetables are processed.

When researchers study the formation of pheophytin in plant material using the techniques of paper chromatography, they find it necessary to run accurate chromatograms on fresh, untreated plant material of the same kind being studied. Can you suggest reasons why this is so? Do you think it would be important to have accurate data from plants that contain little or no pheophytin? Pheophytin is formed in plant material when chlorophyll molecules lose magnesium atoms and gain hydrogen atoms. See if you can relate this knowledge to any of the procedures that follow.

MATERIALS (EACH TEAM)

460 ml petroleum ether
40 ml acetone
10 ea. mortar & pestle or household blender
1 1-qt. screw cap jar
20 small glass containers
with cork stoppers
chromatography jar or
1-gal. wide-mouth mayonnaise jar
chromatographic paper or filter paper
10 scissors
10 mm rulers
10 pencils
10 800 ml beakers
toothpicks
labels
fresh broccoli spears
fresh spinach
stapler
vinegar
sodium bicarbonate
sand

PROCEDURE

Caution: Do not expose petroleum ether, acetone, or alcohol to open flame or to the exposed heating elements of electric hot plates. Work in a well-ventilated room.

1. Place a few fresh spinach leaves in a mortar, along with a pinch or two of clean sand. Add small amounts of acetone while you grind the plant material with the pestle to bring the volume of acetone to approximately 30 ml as the leaves are crushed and ground. After grinding, and when the plant material has settled to the bottom of the mortar, carefully pour 10 ml of the clear green liquid into a 20 ml bottle. Stopper the bottle with a cork stopper, then label it "control". Repeat the same procedure only this time use a fresh broccoli spear. Slice the spear into small pieces prior to grinding in the mortar. Pour the clear green liquid into a second bottle and stopper; label as before.

2. Ask your teacher for a clean piece of chromatographic paper — be certain to handle it only by the edges. Depending on the size of your "developing chamber" (this can be a 1-qt. jar or a 1-gal wide-mouth glass mayonnaise jar), the paper should be cut to the following size:

   5-½" x 7-½" for 1 quart jars
   8" x 11-½" for 1 gallon jars

   Using a lead pencil only, draw a light pencil line across the long axis of the paper approximately one inch from the bottom edge (Fig. 1). Then, draw a second line 12

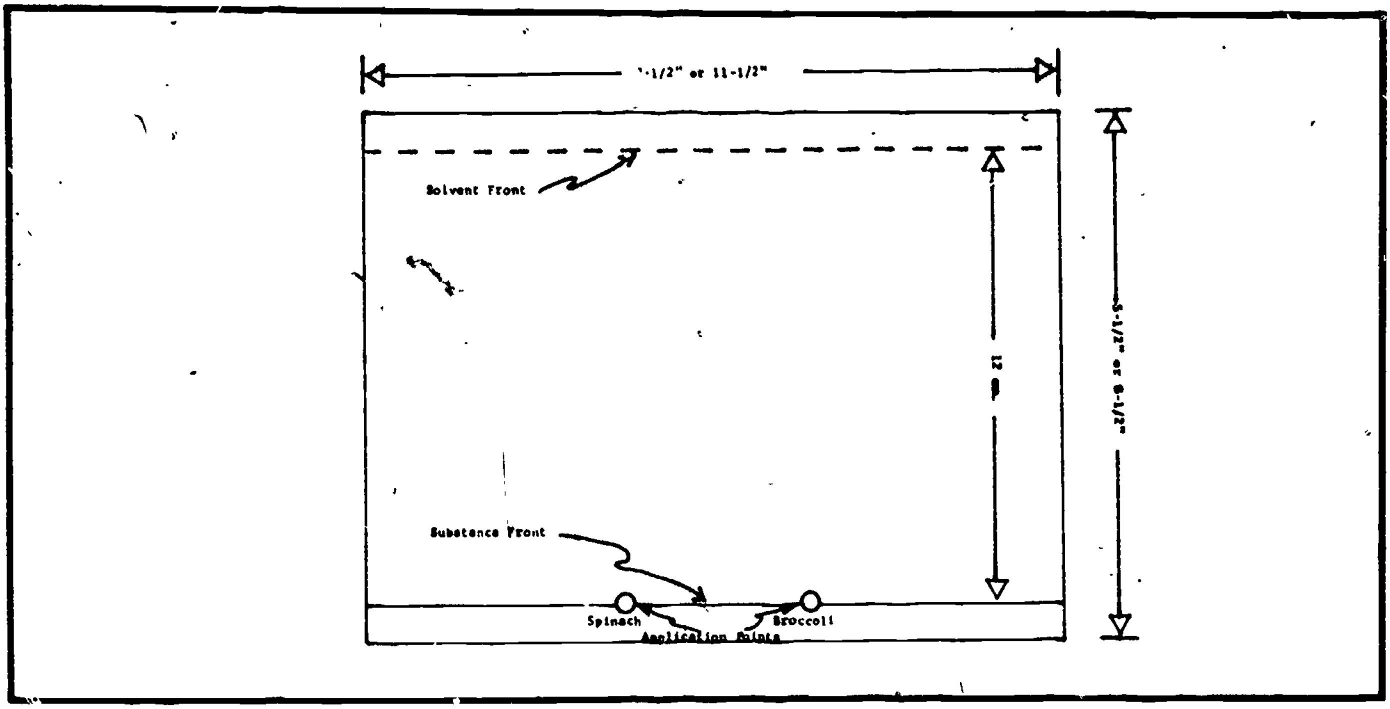

FIG. 1

cm above the first and label this line solvent front. Just below the bottom line, and approximately one-third of the way in on the left side, write the word broccoli. Do the same thing on the right side and write the word spinach. Your paper should now look like the diagram in Fig. 1.

3. Using clean toothpicks, apply 50 very small drops of broccoli extract to the appropriate place on your chromatography paper. Do the same thing with the spinach extract. Try to keep the spots as small as possible at the point of application. When you have applied enough extract, let it dry and form the paper into a cylinder. Staple the edge of the paper in four places as shown in Fig. 2. You are now ready to run your chromatogram.

4. Remove the lid from the developing chamber into which your teacher has placed the solvent mixture, and carefully place the paper cylinder on the bottom of the jar. Immediately replace the lid to prevent solvent fumes from escaping. The jar has been charged with a mixture of 92% petroleum ether and 8% acetone, but less than 1 deep. This solvvent mixture will ascend the paper cylinder. The solvent should be allowed to travel up the paper to the line labeled solvent front. When the solvent has reached this point, quickly remove the paper cylinder from the jar and allow it to air-dry. Remove the staples and place the chromatogram on a clean sheet of paper.

5. Immediately identify the region for each pigment separated. Record in your data table an accurate description of each color observed and, with a pencil, outline each pigment spot. Using a milimeter ruler, measure the distance traveled by each pigment from the point of extract application. Record this information in your

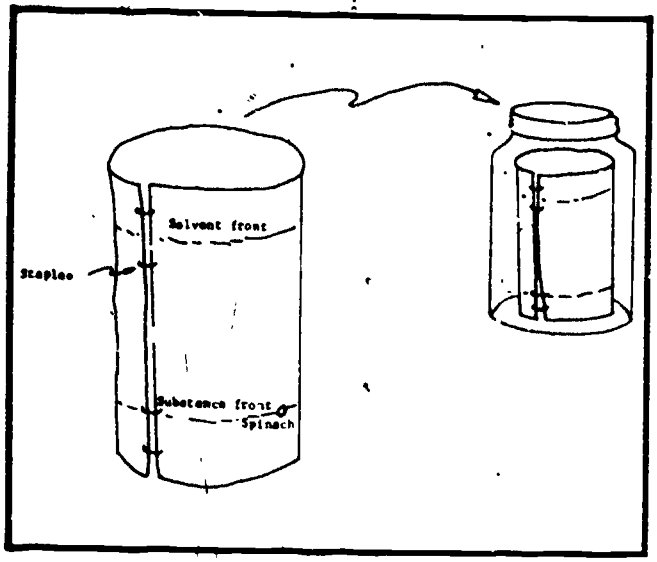

FIG. 2

notebook.

6. In paper chromatogra, , certain substances can be tentatively identified by their R_f value -- the distance which the substance moves up the paper as compared with the distance to which the solvent front moves. The R_f value is calculated as follows:

$$R_f = \frac{\text{Distance moved by substance}}{\text{Distance moved by solvent}}$$

Calculate the R_f values for each of the various pigments on your chromatogram. Compare your data with the information in Table 1.

7. Repeat Steps 1 through 6, only this time treat your fresh plant material as follows:

   (a) Using a cooking time of 15 minutes, boil in separate 800 ml beakers 50 grams of broccoli and spinach in 500 ml of distilled water. Drain the vegetables, cooling them immediately in cold running tap water. Then continue Steps 1 through 6.

   (b) Using a cooking time of 15 minutes, boil in separate 800 ml beakers 50 grams each of broccoli and spinach in 500 ml of distilled water to which 10 ml vinegar has been added. Immediately cool in cold running tap water. Drain the vegetables and continue Steps 1 through 6.

   (c) Using a cooking time of 15 minutes, boil in separate 800 ml beakers 50 grams each of broccoli and spinach in 500 ml of distilled water to which 2 grams of sodium bicarbonate has been added. Drain the vegetables and cool immediately in cold running tap water. Then carry out Steps 1 through 6.

   (d) Following the procedure outlined in Step 3, make applications of each of the four prepared extracts to your chromatography paper. Why would you use the extract from the fresh untreated plant material? Be sure to run one chromatogram for broccoli and one for spinach.

8. When you have calculated the R_f values and have finished recording your data from three chromatograms, use this information as you consider the questions following, while writing up your investigation.

QUESTIONS

1. How many different colors can you see on the chromatograms?
2. Can you tell what the individual pigments are? How?
3. Does a certain pigment always have approximately the same R_f value when chromatographed under identical conditions?
4. Which extract acted as a control? Explain.
5. Can you determine which treatment accel-

Approximate R_f Values of Select Plant Pigments

(Solvent: 92% petroleum ether and 8% acetone)

Pigment	Color	Approximate R_f Value
1. Carotenes	Yellow	.95
2. Pheophytin	Gray-olive green	.6
3. Xanthophyll	Yellow	.45
4. Chlorophyll (a)	Bluish-green	.4
5. Chlorophyll (b)	Yellowish-green	.25

Table 1

erated the degradation of chlorophyll? Which treatment retarded the degradation of chlorophyll? Explain.

6. Based on your observations, which treatment produced the greatest amount of pheophytin? Can you suggest reasons why?
7. If you were a food processor, explain how you might use this information.

EXTENDING THE INVESTIGATION

Try to determine the effects of different cooking times on pheophytin formation in various plant materials different than those you have used.

SUGGESTED READING

Scientific American, Vol. 199 (4), 1960, p. 58, Stein, W. H., and Moore, S., Chromatography. An excellent article dealing with the general principles of chromatography.

Paper Chromatography, Zweig, G. and Whitaker, J. R., 1971. Paper Chromatography and Electrophoresis, Vol. II. Academic Press, New York, 614 pp. Everything you always wanted to know about paper chromatography. A must for any science reference library.

science study aids

are a series of supplementary investigative materials for use in secondary science classes, grades 7 - 12. The materials are based on federal and private research programs. They are written by secondary science teachers working with scientists at research facilities throughout the country. Before being published, they are tested in the laboratory and in classrooms of cooperating teachers.

Several times during the year, new SSA's are developed. If you want to be notified of their availability, request that your name be added to Wordwork's mailing list. Because we cannot provide enough copies for students, we have designed SSA's so that teachers can easily reproduce the student portion for their classes.

We hope that you find the Science Study Aid Series a valuable supplement to your science curriculum. We welcome you comments on the SSA's that you receive.

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