

Chapter 11

Pungency Assessment in Onions

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Introduction

This laboratory exercise is the result of a collaboration between an applied research lab in the College of Agriculture and the teaching program in the Division of Biological Sciences at the University of Georgia. In its current form, this exercise is designed for placement in a two semester sequence of general Biology for Science majors. Parts of this exercise are suitable for inclusion in a nonscience majors Biology course (such as the standard curve and an assay of one onion cultivar). With revision, this exercise could also be used in a Cellular Biology or Plant Physiology course. It is our intent to demonstrate to beginning science students that theoretical lab work can have commercial applications.

This laboratory exercise can be expanded to include the rearing of the onion cultivars or onion allies as part of the experimental protocol. Recipes for the hydroponic solutions and a schematic for the hydroponic growth chambers are included in Appendix A. A schematic for building a commercial onion press are presented in Appendix B. A commercial (industrial grade) juicer can be substituted for the Randle-Brussard Press. The materials listed in the Preparator's Guide (Appendix C) are generally per group of two students -- you can modify the quantities for students to work in groups of 2 or 3, depending on the level/ability of the students.

Student Outline

Introduction

Onions are an important vegetable world-wide, ranking second among all vegetables in economic importance with an estimated value of \$6 billion dollars annually. While onions contribute significant nutritional value to the human diet and have medicinal properties, they are primarily consumed for their unique flavor or for their ability to enhance the flavor of other foods. In 1995, United States per capita

onion consumption was approximately 18 pounds which was a 100% increase from the 1975 figures. While compounds such as sugars and organic acids can contribute to the organoleptic experience, it is a special class of biologically active organosulfur compounds which give onions their distinctive flavor and aroma. Many of these sulfur compounds can be chemically quantified, which is necessary for objectively determining flavor strength as eating just one pungent onion can destroy the ability of a person to test onions further.

Flavor chemistry

Flavor in onions (*Allium cepa* L.) and other vegetable alliums (e.g., garlic, leek, and chives) is dominated by organosulfur compounds arising from the enzymatic decomposition of S-alk(en)yl-L-cysteine S-oxide flavor precursors. There are 3 different flavor precursors in onions: 1-propenyl cysteine sulfoxide which is usually found in the highest concentration; methyl cysteine sulfoxide which is normally found in lesser concentration; and propyl cysteine sulfoxide which is found in the lowest concentration. A fourth precursor, allyl cysteine sulfoxide (the primary flavor precursor of garlic) is found in other vegetable alliums. Differences in flavor intensity within a species are due to differences in the precursors concentrations. The higher the concentration of the flavor precursors, the greater the flavor. Differences in flavor among the vegetable alliums (e.g., why garlic tastes different than onions) results from the presence of the different flavor precursors and the ratio in which they accumulate.

Within intact cells the enzyme alliinase is compartmentalized in the cell vacuole and the flavor precursors are found in the cytoplasm. A reaction, therefore, only occurs when onion tissues are damaged and the enzyme and substrate are brought together as organelles are disrupted. The kinetics of decomposition is different for each specific flavor precursor. The decomposition of 1-propenyl cysteine sulfoxide is almost instantaneous, while the methyl and propyl cysteine sulfoxide decomposition occurs in several minutes. Primary products produced from flavor precursor decomposition include pyruvate, ammonia and chemically unstable sulfenic acids. Among the sulfenic acids is the lachrymator, or tear producing compound, characteristic of onions. The sulfenic acids undergo further rearrangement to form thiosulfinates which are responsible for the characteristic flavor of onions.

Sulfur metabolism in onions

Flavor precursor formation begins with the uptake of sulfate (SO_4^{2-}) by the onion, its reduction to sulfide, and subsequent assimilation into cysteine by light-dependent reactions in the leaves of the plant. Glutathione, a tripeptide of cysteine is then synthesized. This is the starting point of the flavor precursor biosynthetic pathway. The pathways leading to the synthesis of each flavor precursor are not fully understood, although sulfur is known to be transformed through several identifiable peptide intermediates, each unique to a specific flavor precursor.

Factors influencing flavor intensity

To a large extent, the concentration of flavor precursors in onions are determined by the genetics of the cultivar. Onions differ in pungency because cultivars accumulate different amounts of the flavor precursors. The genetic system of onion controls sulfur uptake and the assimilation of the sulfur into the flavor precursors. The genetic regulation of flavor in onions is not understood, but is most likely complex, given the many steps and transformations sulfur goes through prior to becoming a part of the flavor precursors. Each of these steps is controlled by an enzyme which is a product of the plants' genetic system.

The growing environment can greatly influence flavor intensity for any given cultivar. A cultivar with the potential to be mild can be made pungent if grown under conditions conducive for maximum flavor production. Conversely, a pungent cultivar can be made more mild if grown under conditions which favor minimum flavor production. Environments which affect flavor intensity are

sulfate availability, growing temperature, and water availability. The more sulfate available for uptake, the more pungent or flavorful a cultivar will be. Higher growing temperatures and drier growing conditions both result in higher pungency. Conversely, low sulfate availability, low growing temperatures, and moist growing conditions will lower onion flavor intensity.

Measurement of onion flavor

A common assessment of pungency is made by measuring pyruvate, which is formed as a stable primary compound from the enzymatic decomposition of each of the flavor precursors. Pyruvate is produced in a mole for mole relationship with the flavor precursors. It is, however, only an indicator of pungency as pyruvate does not directly contribute to onion flavor.

Objectives

Measuring pyruvate in onions can demonstrate several biological or botanical concepts.

1. Onion is an outcrossing species which undergoes severe inbreeding depression if self pollinated. As a result, it is very hard to stabilize quantitatively inherited traits in onion. Inter-population variation can be assessed to demonstrate the effects of outcrossing on character stabilization for the species. Intra-population onion pungency values can be compared to those of garlic, which is a vegetatively propagated crop and would show less intra-population variation.
2. The effect of sulfate availability on pungency could be demonstrated by growing onions at different sulfur levels. In addition, experiments could be designed to demonstrate nutrient availability and uptake. Different tissues (e.g. leaf, root, bulb) could be analyzed to demonstrate uneven distribution of the flavor compounds within the plant. A discussion of secondary metabolites synthesis and storage in a biennial plant species could be developed.
3. Cultivar differences for pungency could be demonstrated by assessing different onion available in the market place. Normally, at least four different types of onions are available for consumers.

Procedures

This laboratory exercise requires 2 two hour lab periods to complete. During the first lab period, you will learn how to assess the quantity of flavor compounds in the onion cultivars provided by your instructors. Several different cultivars will be evaluated by different student groups. The results will be summarized and compared at the end of the class period. You should concentrate on refining your skills with the pungency protocol. It is essential that you feel comfortable with the construction of a standard curve, can zero (blank) the spectrophotometer, and use the spectrophotometer to obtain a transmittance reading. During the second week, you will be given a choice of several parameters which you can modify in the experimental protocol. You will develop a hypothesis or several related hypotheses, and design an experiment to test your assertions. You will collect data on pyruvate and or soluble sugar concentration(s) and evaluate your experimental results with respect to your hypothesis(es).

Part A. Pyruvic Acid Standard Curve

Following the directions below, obtain the data points necessary to plot a standard curve for pyruvic acid (sodium pyruvate). Record your transmittance data in Table 11.1. Using the data from Table 11.1 and the graph paper provided, plot the pyruvate concentration ($\mu\text{moles/mL}$) on the x-axis and percent transmittance (optical density-O.D.) on the y-axis. Remember that a standard curve is a *best fit curve* -- draw a smooth curve as close to all of the points as possible -- DO NOT connect the points.

Procedures

1. Label five 500 mL volumetric flasks (or graduated cylinders) with the following stock concentrations of pyruvate: **0.1 M**, **0.100 $\mu\text{moles/mL}$** , **0.050 $\mu\text{moles/mL}$** , **0.025 $\mu\text{moles/mL}$** , and **0.010 $\mu\text{moles/mL}$** . Label the 1 L volumetric flask (or graduated cylinder) **0.2 $\mu\text{moles/mL}$** .
2. Make the following solutions as described.
 - 0.1 M sodium pyruvate (0.1 millimole/mL) -- add 1.1 gram of sodium pyruvate salt to 100 mL deionized H_2O in a 500 mL volumetric flask or graduated cylinder.
 - 0.2 $\mu\text{moles pyruvate/mL}$ -- add 2 mL of 0.1 sodium pyruvate to 1000 mL of deionized H_2O in a 1 L volumetric flask or graduated cylinder.
 - 0.100 $\mu\text{moles pyruvate/mL}$ -- add 50 mL of 0.2 $\mu\text{moles sodium pyruvate}$ to 100 mL of deionized H_2O in a 500 mL volumetric flask or graduated cylinder.
 - 0.050 $\mu\text{moles pyruvate/mL}$ -- add 25 mL of 0.2 $\mu\text{moles pyruvate}$ to 100 mL of deionized H_2O in a 500 mL volumetric flask or graduated cylinder.
 - 0.025 $\mu\text{moles pyruvate/mL}$ -- add 12.5 mL of 0.2 $\mu\text{moles pyruvate}$ to 100 mL of deionized H_2O in a 500 mL volumetric flask or graduated cylinder.
 - 0.010 $\mu\text{moles pyruvate/mL}$ -- add 5 mL of 0.2 $\mu\text{moles pyruvate}$ to 100 mL of deionized H_2O in a 500 mL volumetric flask or graduated cylinder.
3. Number 6 spectrophotometer tubes from 1 to 6. Working left to right add 4 mL of each stock solution to one spectrophotometer tube. To Tube #1 add 4 mL of deionized water. This tube will be used as a blank to calibrate the Spectronic-20. Add 4 mL of the 0.2 $\mu\text{moles/mL}$ solution to Tube #2. To Tube #3 add 4 mL of the 0.100 $\mu\text{moles/mL}$ stock solution. To Tube #4 add 4 mL of the 0.050 $\mu\text{moles/mL}$ solution. Add 4 mL of the 0.025 $\mu\text{moles/mL}$ solution to Tube #5. Add 4 mL of the 0.010 $\mu\text{moles/mL}$ solution to Tube #6.
4. Measure the percent transmittance for each of the pyruvate standards. Record the data in Table 11.1.
5. Plot the data in Table 11.1 on the graph paper provided (Figure 11.1). Plot the pyruvate concentration ($\mu\text{moles/mL}$) on the x-axis and percent transmittance (optical density -O.D.) on the y-axis. Construct a standard curve to be used in Part C.

Table 11.1. Percent transmittance for known concentrations of pyruvic acid (sodium pyruvate).

<i>Spec Tube #</i>	<i>Tube #1</i>	<i>Tube #2</i>	<i>Tube #3</i>	<i>Tube #4</i>	<i>Tube #5</i>	<i>Tube #6</i>
pyruvate concentration (μmoles/mL)	blank	0.200	0.100	0.050	0.025	0.010
percent transmittance						

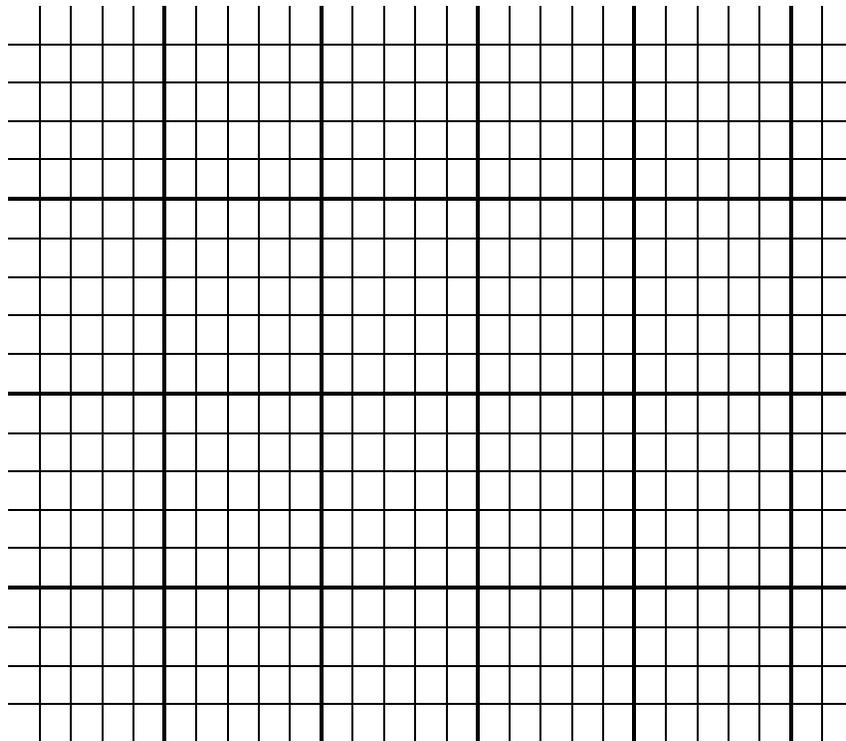
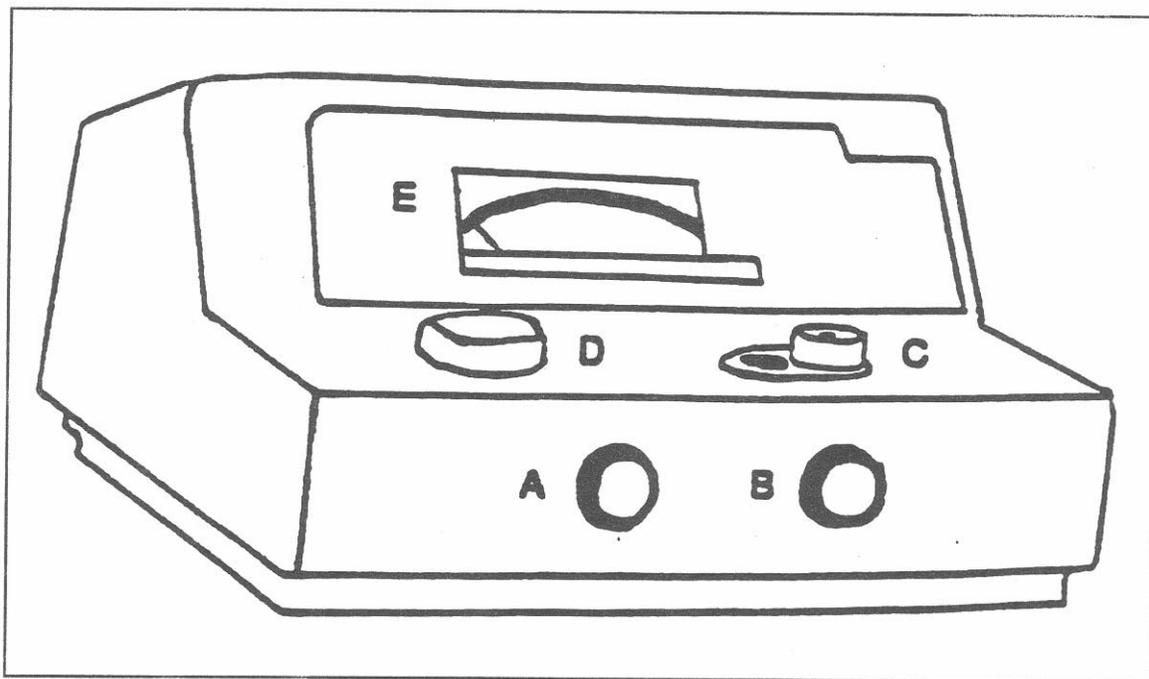


Figure 11.1. Graph paper for plot of pyruvate concentration vs. transmittance.

Operation of the Spectronic 20 (Figure 11.2)

- a. Turn on the instrument with the amplifier control knob (A). Allow at least 10 minutes for the instrument to warm up.
- b. Turn the wavelength control knob (C) until 420 nm appears in the window next to the wavelength control knob.
- c. Zero the instrument by turning the amplifier control knob (A) until the meter needle in (E) reads 0% transmittance. Be sure the sample holder cover (D) is closed when you zero the instrument. Do not zero the instrument with a test tube in the sample holder.
- d. Place the blank (Tube #1 containing 4 mL of deionized water) into the sample holder and close the cover. Adjust the instrument to zero O.D. (optical density or absorbance) or 100% transmittance by turning the light control knob (B).
- e. Remove the blank tube. Insert your sample tube into the sample holder of the spectrophotometer. Make your reading from the transmittance scale on the meter (E). Note that this scale is logarithmic.
- f. Repeat steps d and e, to make further measurements. If you change the wavelength control knob (C), you must zero the instrument again.

**Figure 11.2.** Spectronic 20.

Part B. *Allium* Sample Preparation

In this portion of the laboratory, you will prepare 2 different *Allium* samples, an extract of fresh tissue and an extract of microwaved tissue. The microwaved sample represents a control for other organic compounds.

Procedures

1. In a 1000 mL beaker, blend wedges of the Granex onion bulb (or other *Allium*) with an equal volume of deionized water. For example, blend 20 g of onion with 20 mL of water.
2. Squeeze the sample with the Randle/Brussard press or commercial juicer.
3. Using a refractometer, estimate the soluble sugar content of the *Allium* sample. Place a drop of juice on the refractometer's prism. Point the refractometer toward a light source (artificial or natural). When you look through the eyepiece, an incremental measurement scale should be visible. The numbers on the scale represent the percentage concentration of soluble sugars in the onion extract.

Amount of soluble sugar present _____

4. Transfer the mixture to a 600 mL beaker and cover with a watch glass.
5. Allow the onion/water mixture to sit for 10 minutes.
6. Stirring the solution to suspend onion pieces, pipette 0.5 mL of the onion slurry into a large test tube.
7. Add 1.5 mL of 5% trichloroacetic acid (TCA) to the onion/water solution in the test tube. Cover the top of the test tube with Parafilm and vortex.
8. Allow the preparation to sit for at least 1 hour. Remove the Parafilm and add 18 mL of deionized water to the test tube. Seal the test tube with Parafilm and vortex.
9. This dilution should yield readings between 20% and 80% transmittance. For Granex variety onions, a 1 to 10 dilution should produce a pyruvate solution within the transmittance range above. For other *Allium* species such as chives, garlic, leeks, and scallions, additional dilution will be required. If more pungent, long day onion varieties are used, additional dilutions may be necessary to produce a solution which will be within 20% to 80% transmittance.
10. Prepare a control (background) sample following the protocol above. Heat the onion wedges by microwaving the onion pieces on high for 7 minutes. Process the control sample as described above.

Part C. Pyruvic Acid Determination

In this portion of the laboratory, you will determine the pyruvate concentration of a Granex onion. Using the standard curve constructed in *Part A*, you will estimate the pyruvate concentration for the onion by reading the pyruvate concentration value which corresponds to the observed percent transmittance measurement from your standard curve.

Procedures

1. Label a small test tube: **Tube #7**. Transfer 1 mL of the fresh onion slurry/5% TCA solution into the small test tube.
2. Label a small test tube: **Tube #8**. Transfer 1 mL of the microwaved onion slurry/5% TCA solution into the small test tube.
3. Add 1 mL of 0.0125% 2,4-dinitrophenylhydrazine (2,4-DNPH) reagent and 1 mL of deionized water to each test tube. *2,4-DNPH is very toxic; always wear gloves when handling this reagent.* Alert your lab instructors immediately if any of the reagent or onion mixture containing 2,4-DNPH is spilled. Cover the test tubes with Parafilm and vortex.
4. Place the test tubes (fresh tissue and microwaved tissue samples) in a 37°C water bath. Allow the mixture to react for 10 minutes.
5. Remove the Parafilm from each tube. Add 5 mL of 0.6 N NaOH to each tube. Seal tubes #7 and #8 with Parafilm and vortex.
6. Zero the spectrophotometer following the procedures outlined in *Part A*.
7. Pipette 5 mL of one of the solutions (Tube #7-fresh tissue; Tube #8-microwaved tissue) into a spectrophotometer tube. Read the percent transmittance for the sample at 420 nm. Record the data for the sample in Table 11.2. Repeat the process for the other tube. Use the standard curve from *Part A* to estimate the pyruvate concentration for both samples.

Table 11.2. Pyruvate concentration estimates for fresh and microwave-heated *Allium* tissue.⁴ Values are estimates from the standard curve on in Figure 11.1.

<i>Spec Tube #</i>	<i>Tube #7</i>	<i>Tube #8</i>
percent transmittance		
pyruvate concentration ($\mu\text{moles/mL}$)⁴		

Calculations

Pungency is a function of $\mu\text{moles pyruvic acid/gram}$ of tissue. To calculate the $\mu\text{moles pyruvate/gram}$ tissue you must consider how the original sample was diluted:

protocol step	dilution factor
1:1 blend of tissue/DI H ₂ O	2 X
0.5 mL tissue slurry: 1.5 mL 5% TCA (20 g tissue: 20 mL TCA)	2 X
dilution factor (Granex) 2 mL solution: 18 mL DI H ₂ O ⁵	<u>10 X</u>
TOTAL DILUTION	40 X

⁵ For other *Allium* species where additional dilution is necessary, determine the total dilution as a function of the 1:10 ratio above. For instance, if the solution was further diluted 1:1, the dilution factor for this step would be $10 \times 2 = 20$.

$\mu\text{moles pyruvate/g tissue} = \mu\text{moles/mL (read from standard curve--Table 11.2)} \times \text{Total Dilution}$

Using the equation above and the data from Table 11.2, calculate the pyruvate concentration ($\mu\text{moles/g}$) in the fresh and microwave-heated samples. Record your values in Table 11.3 below.

Table 11.3. Calculated values for pyruvate concentration and enzymatic development for the *Allium* tissues.

<i>Tissue Type</i>	<i>Fresh Tissue</i>	<i>Microwave-heated Tissue</i>
$\mu\text{moles pyruvate/mL}$ (refer to Table 11.2)		
$\mu\text{moles pyruvate/g tissue}$ (calculated)		
Enzymatic pyruvate development = $\mu\text{moles/g fresh tissue} - \mu\text{moles/g microwave heated tissue} =$ _____ $\mu\text{moles/g}$		

Using the calculated $\mu\text{mole/g}$ tissue values and the equation at the bottom to Table 11.3, determine the total enzymatic pyruvate development for your particular Granex cultivar. Compare your results with those of your classmates.

Table 11.4. Enzymatic pyruvate activity for various onion cultivars.

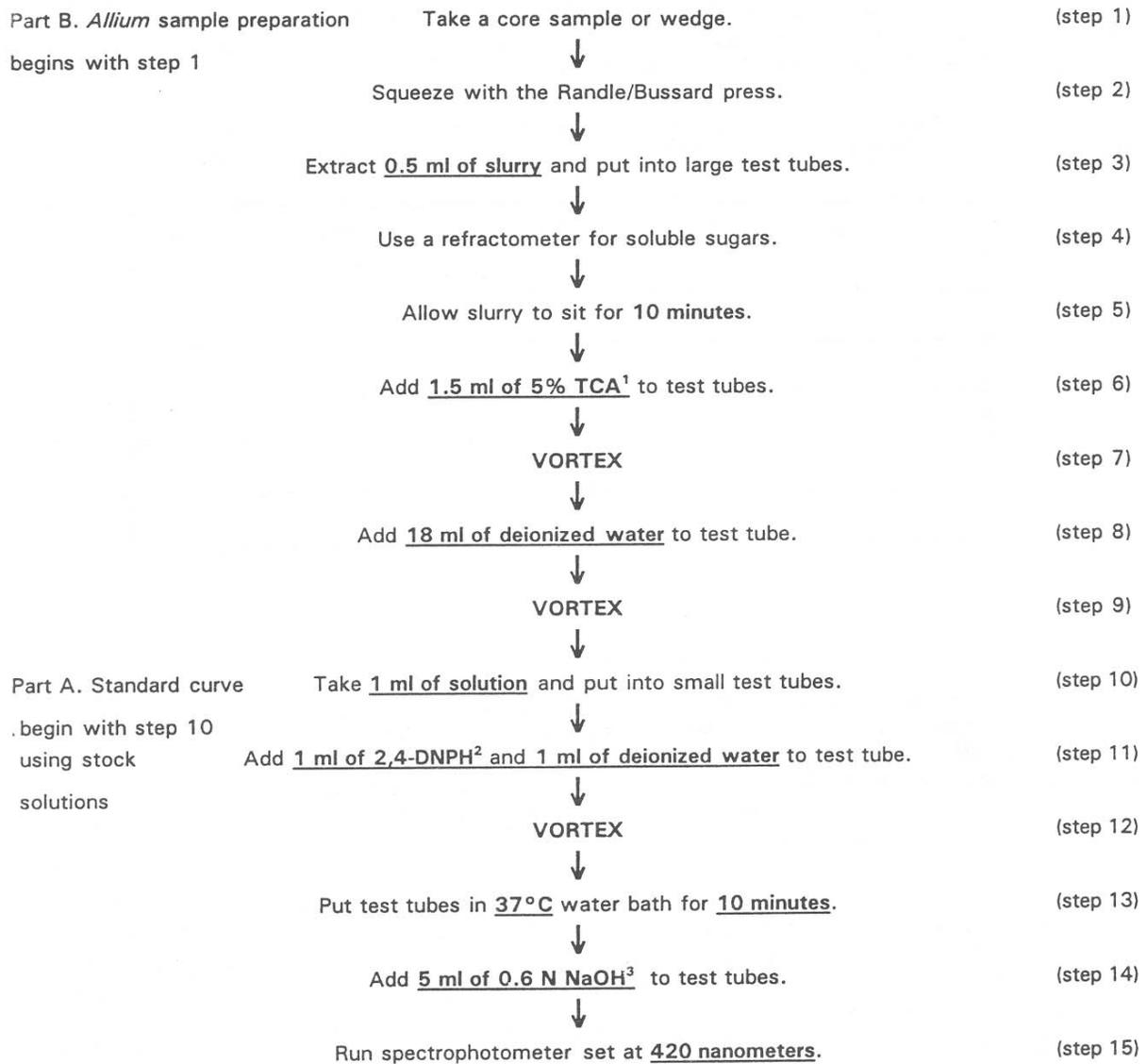
Note: If more than one estimate is obtained for a cultivar, report the average of all data for that cultivar.

Onion Cultivar	Enzymatic Pyruvate Development $\mu\text{moles pyruvate/g tissue}$

Thought Questions

1. What environmental factors would affect the quantity of flavor precursors in *Allium*?
2. Would expect the flavor precursors to be present in differing concentrations in distinct regions of the *Allium* plant?
3. In which part of the plant do you think the flavor precursors are concentrated?
4. There are two types of commercial onions -- long day and short day plants. Short day plants require a short photoperiod (long night) in order to produce a large bulb. Short day varieties are grown in the southern United States in areas such as Georgia (Vidalia cultivar) and Texas (Texas sweet cultivar). Long day onions have longer photoperiod requirements (short nights) than their southern counterparts. Long day cultivars such as Walla Walla (Washington state) are grown in areas farther north. How might day length and time to maturity affect the concentration of flavor precursors in onions? Explain your answer -- relate photoperiod to photoperiod phytochromes

P_{fr} to P_r , environmental temperatures, and moisture patterns (rain versus snow). Remember that P_{fr} refers to a plant pigment called phytochrome which absorbs light in the far-red region (730 nm). P_r is a plant phytochrome which absorbs light at 660 nm in the red region of the spectrum. In many alliums, P_{fr} stimulates underground bulb formation while P_r will produce leaf blade growth. It is the P_r to P_{fr} ratio which determines whether the plant invests energy in bulb formation or above-ground growth.



¹ 5% TCA
Dissolve 50 grams trichloroacetic acid in distilled water to make 1000 ml.

² 2,4-DNPH (0.125% DNPH in 2 N HCl)
Dissolve 0.125 grams of 2,4-dinitrophenylhydrazine in 1000 ml of 2 N HCl solution.
To make 2 N HCL solution, dilute 228 ml of 37.8% HCl to 1000 ml of distilled water.

³ 0.6 N NaOH
Dissolve 24 grams of NaOH in distilled water to make 1000 ml.

Figure 11.3. Flow chart for onion pungency analysis.

Student Designed Experiments

Prior to this laboratory period, you will develop a hypothesis relating to the *Allium* flavor precursors. Using the protocol which you mastered during the last laboratory period, you will evaluate your hypothesis based on your interpretation of the data you collect during this lab period. You will need to refer to the standard curve in *Part A* to estimate the concentration of pyruvate in mmoles/mL. Calculate the enzymatic pyruvate development for the tissues you elect to use. It is important that you remember to compare a control for each treatment group (microwave-heated tissue sample).

Choose one of the independent variables from the list below:

1. **variety of *Allium*** -- chives, leeks, scallions, and garlic
2. **sulfur concentration in the growth medium** -- several bulbs of one cultivar are grown in Hoagland's solution with different sulfur concentrations (high, medium, and low)
3. **type of plant tissue** (vegetative [stems or bulb] or reproductive) -- onion bulbs with the top of the plant and flowers intact
4. **cultivation temperature** -- several bulbs of one cultivar grown in Hoagland's solution at 4 different environmental temperatures (15°C, 20°C, 25°C, and 30°C).

Let your lab instructors know in a week in advance which one of these variables you will be using. This will assure that you will have an adequate amount of plant tissue available.

Hypothesis

Write a hypothesis for the variable you and your lab partner have chosen. Draft the hypothesis as an "operational" statement. Relate your predictions of experimental outcome to the actual measurements you make. For example, if the phosphate concentration in mg/mL in the growth medium is increased (independent variable), then the soluble starch concentration (mg/mL) would decrease (dependent variable). In this example, the hypothesis includes the actual units of measurement for both variables of interest.

Test of Hypothesis (to be completed before coming to class)

Write a flow chart outlining the experiment that you will perform. Include any equipment, settings for the equipment, amounts of reagents to be used, and time constraints for each step. Use the outline of Onion Pungency Analysis in the previous section as a model for your flow chart.

Results

Construct a data table based on your experimental design and record the data.

Conclusions

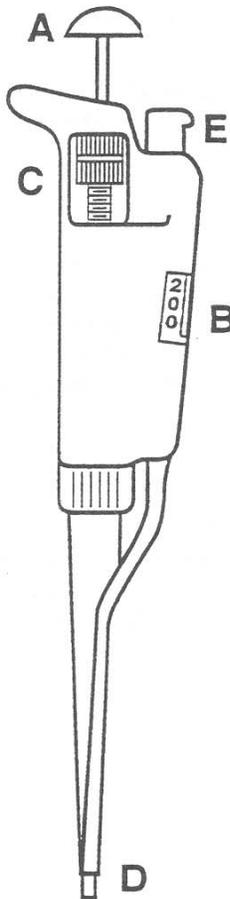
1. Do the data collected support or fail to support your hypothesis?
2. Identify any possible sources of error in your experimental design.
3. Why is it important to use controls in an experiment?
4. What did you use as a control(s) in your experiment?
5. How many replicates did you use in your experiment?
6. How might sample size and replicate number affect your ability to generalize your

experimental results to the larger population (onion cultivar type, etc.) from which your samples were derived?

DIRECTIONS FOR USING PIPETMAN

1. The size of the Pipetman (in microliters) is indicated on the top of the plunger (A): 1000 (blue), 200 (yellow), and 20 (yellow).
2. The volume to be dispensed is displayed in window (B), and set by turning knob (C).

For this lab, the amount will be pre-set.
3. Obtain a sterile tip by opening the box containing tips and inserting the Pipetman (D) into a tip (color-coded to Pipetman), to avoid contamination, **DO NOT TOUCH TIPS.**
4. Plunger (A) has 2 stops. The first is for uptake of liquid and the second is for dispensing.
5. **TO TAKE UP LIQUID:**
Push plunger (A) to first stop.
Insert Pipetman into liquid.
Slowly release plunger, to avoid air bubbles.
6. **TO DISPENSE LIQUID from Pipetman:**
Push plunger (A) to second stop, all the way down past resistance of first stop.
7. To remove tip, push small plunger (E).



DIRECTIONS FOR USING BULB PROPIPETTE

Attaching bulb to pipette:

Squeeze (A) and bulb simultaneously; bulb collapses.
Hold pipette firmly near narrow cylindrical end.
Insert pipette into (S)-end of bulb.

Sucking liquid into pipette:

Squeeze (S) lightly; liquid enters pipette.

Removing liquid from pipette:

Squeeze (E) lightly to express (=remove) liquid.
To remove last bit of liquid from blow-out pipettes, squeeze (A) and (E).

(S) is pinched to suck up liquid and **(E)** is pinched to release the liquid. If a blow-out pipette is used, **(A)** must also be squeezed to remove all liquid.

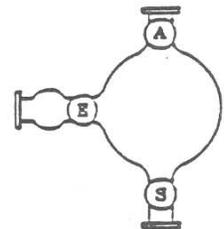


Figure 11.4. Use of pipetman.

Figure 11.5. Use of propipette.

Hydroponic Schematic Diagram

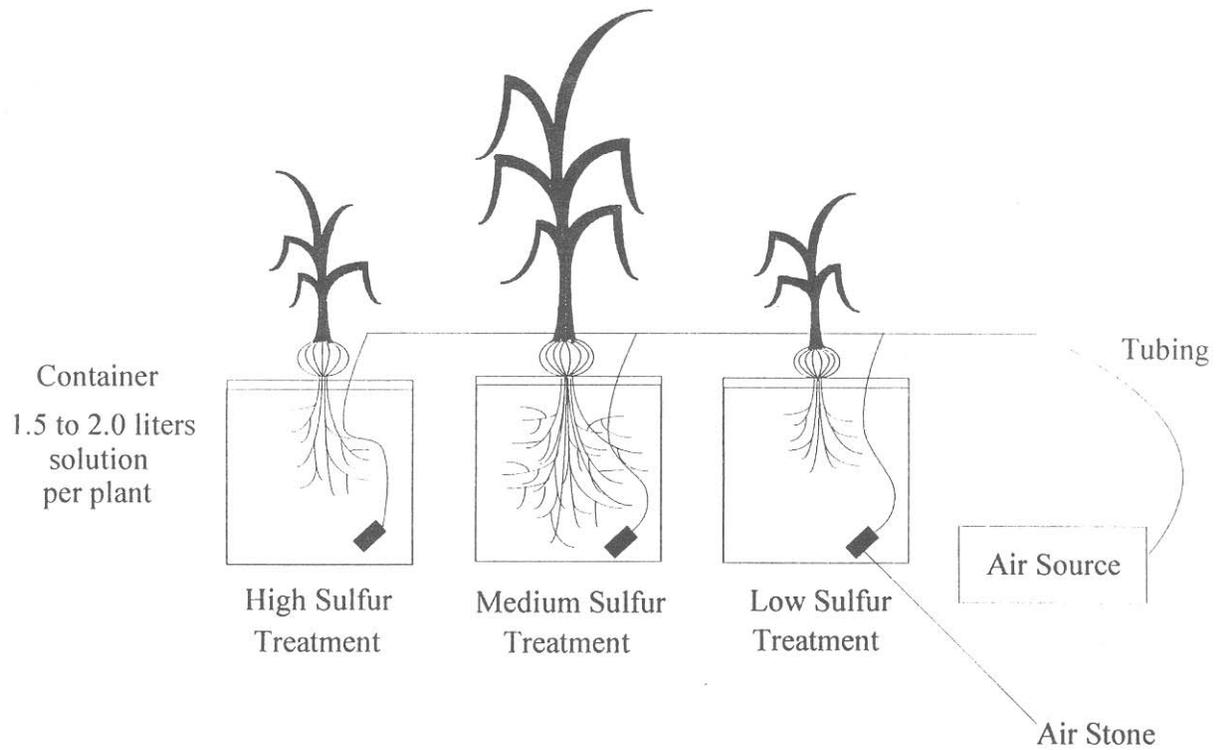


Figure 11.6. Hydroponic growth of onions.

Acknowledgments

The authors would like to thank Dave Kopsell for preparing the computer generated hydroponics schematic. Special thank are also due to Dean Kopsell and Rachel Snyder for testing the laboratory protocol and editorial assistance.

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Appendix A

Nutrient Recipes to Vary Sulfur in a Nutrient Solution

In order to see an effect on the flavor system of onions, garlic, leeks, or chives with the recipes outlined below, the containers holding the solutions should provide approximately 1.5 to 2.0 liters of solution per plant. Volumes greater than this will negate the effect of sulfur on flavor development.

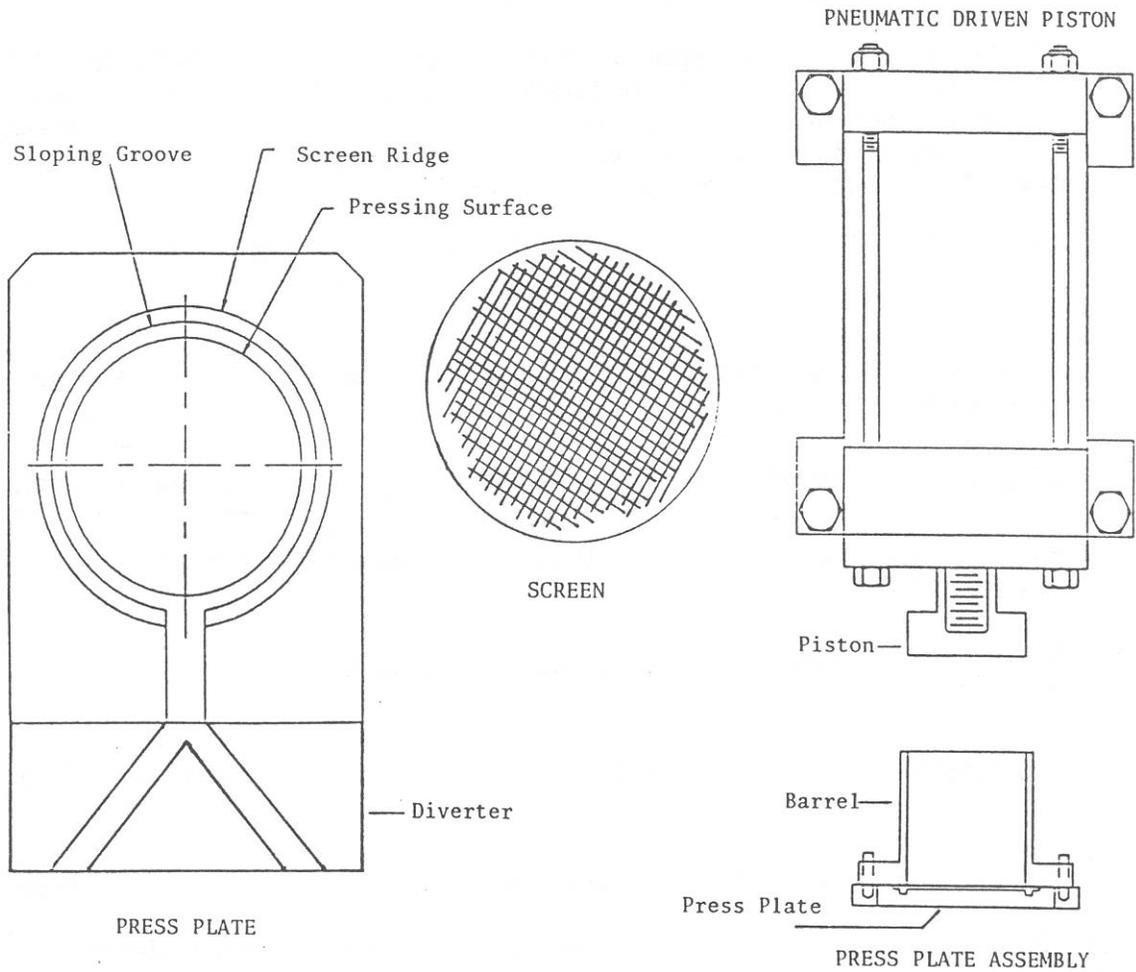
<i>Salt</i>	<i>Stock Solution (g/L)</i>	<i>Final Solution (mL/L)</i>		
		<i>High S</i>	<i>Med S</i>	<i>Low S</i>
Ca(NO ₃) ₂ •4H ₂ O	236.2	2.0	2.0	2.0
KNO ₃	101.1	3.0	3.0	3.0
NH ₄ H ₂ PO ₄	115.0	0.5	0.5	0.5
MgSO ₄ •7H ₂ O	246.5	1.0	0.5	0.1
MgCl ₂ •6H ₂ O	203.3	0.0	0.5	0.9
Fe (chelated)	10.0	0.5	0.5	0.5
Microelements (combined)				
H ₃ BO ₃	2.86			
MnCl ₂ •4H ₂ O	1.81			
ZnSO ₄ •7H ₂ O	0.22			
CuSO ₄ •5H ₂ O	0.08			
H ₂ MoO ₄ •H ₂ O	0.02			

The iron source can be either Sprint 330 (Ciba) or Sequestrene 330, each of which contain 10% chelated iron.

The microelements are made up together as a stock solution and then added to the final solution in the amounts above. The micronutrients are available from Grace-Sierra and are marketed under name Micromax™.

Appendix B
Schematics for the Randle-Brussard Press

The schematic plans for the commercial press follow. A commercial juicer can be substituted for the Randle-Brussard Press.



Appendix C
Preparator's Guide

Reagents for pungency analysis

1. 5% TCA -- add 100 grams trichloroacetic acid to 2000 mL of deionized H₂O.
2. 2 N HCl -- add 384 mL HCl to 2000 mL deionized H₂O (add water to acid; use graduated cylinder to measure HCl).
3. 2,4-dinitrophenylhydrazine (2,4-DNPH)⁴ -- 0.0125% in 2 N HCl, weigh 0.129 grams of 2,4-DNPH and dissolve in 1000 mL of 2 N HCl.
⁴ 2,4-DNPH is very toxic; prepare the stock solution under a hood; weigh the 2,4-DNPH in a polystyrene weigh boat, rinse the powdered reagent into 1000 mL volumetric flask by transferring some of the 2 N HCl into a wash bottle. The volumetric flask should contain at least 500 mL of 2 N HCl before placing it on a hot plate/stirrer. Stir the solution slowly on **LOW** heat until the 2,4-DNPH goes into solution.
4. 0.6 N NaOH -- dissolve 48 grams of NaOH in 2000 mL of deionized H₂O.

Standards

5. 0.1 M sodium pyruvate (0.1 millimole/mL) -- add 1.1 gram of sodium pyruvate salt to 100 mL deionized H₂O.
6. 0.2 μmoles pyruvate/mL -- add 2 mL of 0.1 sodium pyruvate to 1000 mL of deionized H₂O.
7. 0.100 μmoles pyruvate/mL -- add 50 mL of 0.2 μmoles sodium pyruvate to 100 mL of deionized H₂O.
8. 0.050 μmoles pyruvate/ mL -- add 25 mL of 0.2 μmoles pyruvate to 100 mL of deionized H₂O.
9. 0.025 μmoles pyruvate/mL -- add 12.5 mL of 0.2 μmoles pyruvate to 100 mL of deionized H₂O.
10. 0.010 μmoles pyruvate/mL -- add 5 mL of 0.2 μmoles pyruvate to 100 mL of deionized H₂O.

NOTE: Percent transmittance readings for the standards at 420 nm should similar to those in the following table.

pyruvate concentration (μmoles/mL)	blank	0.200	0.100	0.050	0.025	0.010
percent transmittance	100 %	54 %	72 %	81 %	91 %	98 %

Equipment for Pungency Analysis

For each pair of students:

Week 1

Spectronic-20 spectrophotometer (300-600 nm range; available from *Carolina Biological Supply* or *Ward's Biology* -- any spectrophotometer within this range will work)

refractometer (1-2 per class of 30 students) -- available from Forestry, Aquaculture, and some biological supply catalogs.

2 test tube racks (wire or acrylic)

spectrophotometer test tube cuvettes (10 per 2 students)

water bath-37°C (2 water baths for class of 30 students)

25 mL test tubes (10)

Parafilm

scissors

test tube holder

100-1000 µl micropipette

rack of sterile micropipette tips (101-1000 µl)

vortex (8-9 per class of 30 students)

25 mL volumetric pipettes (3 per student)

2 1000 mL beakers

2 600 mL beakers

2 watch glasses (large enough to cover 600 mL beakers)

500 mL volumetric flask or graduated cylinder (5 per group of 2 students)

1 L volumetric flask or graduated cylinder (1 per group of 2 students)

disposable weigh boats

Pipette aid or Propipette bulb to fit 25 mL volumetric pipettes

Randle/Bussard press (contact Dr. Bill Randle, University of Georgia, (706) 542-2471; wrandle@uga.cc.uga.edu, for information on where to purchase the press)

Granex variety onions

scalpel

metric scale (at least 0.5 grams - 100 gram capability)

Week 2

same equipment as above

Living material

variety of other *Allium* species -- chives, leeks, scallions, and garlic

several bulbs of one cultivar grown in Hoagland's solution (4 different sulfur concentrations)

onion bulbs with the top of the plant and flowers intact

several bulbs of one cultivar grown in Hoagland's solution at 4 different environmental temperatures

Appendix D
Sources for Materials

Chemicals for Pyruvate Analysis

	Catalog numbers		
	<i>Fisher</i>	<i>Sigma</i>	<i>ICN</i>
trichloroacetic acid	A322-500	T6399	152592
hydrochloric acid	A144-500	H7020	-----
--			
2,4-dinitrophenylhydrazine	-----	D2630	150122
sodium hydroxide	S318-500	S0899	153495
pyruvic acid-sodium salt	O4376-100	P2256	102926

Vendors

Carolina Biological Supply
2700 York Road
Burlington, North Carolina 27215-3398
Phone: 1-800-334-5551
Fax: 1-800-222-7112

Fisher Scientific
PO BOX 4829
Norcross, Georgia 30091
Phone: 1-800-766-7000

Grace-Sierra (for micronutrients)
1001 Yosemite Drive
Milpitas, California 95035

ICN Biomedicals
3300 Hyland Avenue
Costa Mesa, California 92626
Phone: 1-800-854-0530

Sigma Chemical Company
PO BOX 14508
St. Louis, Missouri 63178
Phone: 1-800-325-3010

Ward's Biology
PO BOX 92912
Rochester, New York 14692-9012
Phone: 1-800-962-2660
Fax: 1-800-635-8439