



## Which Orange Juice Has the Most Vitamin C?

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### Objective

The goal of this project is to determine which orange juice has the most vitamin C: home-made fresh-squeezed, premium not-from-concentrate, or juice made from frozen concentrate.

### Introduction

In this project, you will determine the amount of vitamin C in various types of orange juice. You'll use a method called *titration*, which is a common technique in chemistry. Titration is a way to measure the unknown amount of a chemical in a solution (the *titrant*) by adding a measured amount of a chemical with a known concentration (the *titrating solution*). The titrating solution reacts with the titrant, and the endpoint of the reaction is monitored in some way. The concentration of the titrant can now be calculated from the amount of titrating solution added and the ratio of the two chemicals in the chemical equation for the reaction.

Let's go through the titration process with a specific example: the titration of vitamin C by iodine. The chemical name for vitamin C is *ascorbic acid*. When iodine and ascorbic acid are combined in solution, a chemical reaction takes place. In this chemical reaction, the ascorbic acid molecule loses electrons, which are transferred to the iodine molecule. Chemists call this type of reaction an *oxidation/reduction* reaction (or *redox* reaction for short). The ascorbic acid is oxidized to dehydroascorbic acid, and the iodine is reduced to iodide ions. Oxidation-reduction reactions always occur in pairs like this. The molecule that loses electrons is *oxidized*, and the molecule that accepts the electrons is *reduced*.

So how can you use the iodine-ascorbic acid reaction to determine the amount of ascorbic acid (vitamin C)? If you start with a known concentration of iodine, and carefully measure the amount of the iodine solution that you add, you can calculate how much ascorbic acid was present. How do you know when the iodine-ascorbic acid reaction is complete? You add an indicator to the solution. In this case, the indicator is soluble starch. When iodine reacts with starch, it turns the solution a blue-black color. If ascorbic acid is present in the solution, iodine will react with it, and not with the starch, so the solution will not change color. However, once all of the ascorbic acid has been oxidized, added iodine will be free to react with the starch, producing a distinct color change.

Which do you think will have more vitamin C (ascorbic acid): freshly picked oranges, or oranges that were picked one or two weeks ago? Does it matter if the oranges are stored at room temperature or in a refrigerator? You can find out for yourself with this project!

### Terms, Concepts and Questions to Start Background Research

To do this project, you should do research that enables you to understand the following terms and concepts:

- Titration
- Stoichiometry
- Vitamin C
- Iodine

More advanced students should also study:

- Oxidation-reduction (redox) reactions
- Redox potential

### Questions

- What happens when iodine is added to a starch solution?
- What happens when iodine is added to a starch solution that also contains vitamin C?

### Bibliography

- For a good, basic reference on chemistry, see: Andrew Rader Studios, 1997–2007. "Chem4Kids," Chem4Kids.com [accessed July 18, 2007] <http://www.chem4kids.com>.
- The Materials Safety Data Sheet (MSDS) for Lugol's solution has important chemical safety information: Home Science Tools, n.d. "MSDS for Lugol's Iodine Solution," Home Science Tools [accessed July 18, 2007] <http://www.hometrainingtools.com/catalog/file/104/CH- IODINE.pdf>.
- This project is based on:
  - University of Canterbury, n.d. "Determination of vitamin C Concentration by Titration," Science Outreach Programme, College of Science, University of Canterbury, Christchurch, New Zealand [accessed July 18, 2007] [http://www.outreach.canterbury.ac.nz/chemistry/documents/vitaminc\\_iodine.pdf](http://www.outreach.canterbury.ac.nz/chemistry/documents/vitaminc_iodine.pdf), and
  - Ganong, B., n.d. "Determination of Vitamin C in Orange Juice," Mansfield University [accessed July 18, 2007] <http://faculty.mansfield.edu/bganong/biochemistry/vitaminc.htm>.
- Another useful reference on the vitamin C titration procedure is: Helmenstine, A.M., 2007. "Vitamin C Determination by Iodine Titration," About.com: Chemistry [accessed July 18, 2007] <http://chemistry.about.com/od/demonstrationexperiments/ss/vitctitration.htm>.
- More advanced students should do background research on stoichiometry and oxidation-reduction (redox) reactions. Here is a helpful review of high school chemistry that can be a good starting point:
  - Shodor Education Foundation, Inc., 1998a. "Stoichiometry," UNC-Chapel Hill Chemistry Fundamentals Program [accessed July 18, 2007] <http://www.shodor.org/unchem/basic/stoic/index.html>.
  - Shodor Education Foundation, Inc., 1998b. "Redox Reactions," UNC-Chapel Hill Chemistry Fundamentals Program [accessed July 18, 2007] <http://www.shodor.org/unchem/advanced/redox/index.html>.

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### Project Summary

<a href="#">Difficulty</a>	7 - 8
<a href="#">Time required</a>	Long (a couple of weeks)
<a href="#">Prerequisites</a>	Ideally you'd have your own citrus tree with ripe fruit for this project. The second-best option is to use citrus fruit from a store.
<a href="#">Material Availability</a>	Specialty items
<a href="#">Cost</a>	Average (\$50 - \$100)
<a href="#">Safety</a>	Adult supervision required

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## Materials and Equipment

To do this experiment you will need the following materials and equipment:

- Samples of three different kinds of orange juice:
  - a. home-made fresh-squeezed (which means you'll need to buy some oranges),
  - b. premium not-from-concentrate juice (e.g. Tropicana® or Florida's Natural®), and
  - c. made from frozen concentrate (following instructions on the concentrate can).
- Juicer for extracting juice from oranges (manual or electric is fine)
- Cheesecloth
- Vitamin C tablets
- Distilled water
- Transfer pipettes
- Masking tape
- Permanent marker
- Small funnel (do not use for food after using it for chemistry)
- The following items can be ordered from [Science Kit & Boreal Laboratories](#) or [Home Science Tools](#):
  - Chemical safety goggles
  - Lab apron
  - Rubber (latex) gloves
  - Iodine solution, use *one* of the following:
    - Lugol's iodine solution (part # CH-iodine from Home Science Tools) *or*
    - iodine solution for starch test (part # WW9704606 from Science Kit & Boreal Laboratories).
  - Soluble starch
  - 50 mL graduated cylinder
  - 250 mL graduated cylinder
  - 500 mL graduated cylinder
  - 50 mL Erlenmeyer flask
  - 50 mL buret
  - Ring stand
  - Buret clamp
  - Plastic transfer pipettes (or eyedropper) for adding drops of starch indicator solution
- Electronic kitchen balance (accurate to 0.1 g)
- Glass jars for iodine (300 mL) and starch solutions.
- Optional: you can obtain more accurate results if you use a 250 mL volumetric flask for making your vitamin C standard solution.

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## Experimental Procedure

**Note:** iodine solution is poisonous. Avoid skin and eye contact. See the Materials Safety Data Sheet for complete information. Wear chemical safety goggles and rubber gloves when handling the concentrated solution. Iodine solution will stain clothing; a lab coat is also recommended. For more information on how to properly use a balance and different titration techniques see [Chemistry Lab Techniques](#).

1. Do your background research so that you are knowledgeable about the terms, concepts, and questions, above.
2. Wear gloves, chemical safety goggles, and a lab coat or apron when using the iodine solutions in this experiment.
3. Dilute the Lugol's solution 1:10 in distilled water to make your iodine titration solution.
  - a. Pour the 30 mL Lugol's solution into the 500 mL graduated cylinder.
  - b. Add enough distilled water to bring the total fluid volume to 300 mL and mix.
  - c. Store the solution in a clean, tightly covered glass jar that is clearly labeled. Store it in a location that is protected from light.
4. Make a starch indicator solution.
  - a. This can be anywhere from 0.5 to 1.0%. The exact amount of starch is not critical.
  - b. For a 0.5% solution, add 0.25 g of soluble starch to 50 mL of near-boiling distilled water.
  - c. Stir to dissolve, and allow to cool.
  - d. When cool, store the starch solution in a clean, tightly covered glass jar that is clearly labeled.
5. Make a fresh vitamin C standard solution (1 mg/mL). Do this on each day that you make vitamin C measurements from oranges.
  - a. You will use this solution to "standardize" your iodine titration solution. You will measure how much of your iodine solution it takes to oxidize a known amount of vitamin C. You can then use your iodine titration solution to determine the amount of vitamin C from test samples of juice from oranges.
  - b. Crush a 250 mg vitamin C tablet, and dissolve it in 100 mL of distilled water.
  - c. Pour into a graduated cylinder and add distilled water to bring the total volume to 250 mL.
6. Titrate 25 mL of vitamin C standard solution.
  - a. Use a clean 25 mL graduated cylinder to measure 20 mL of vitamin C standard solution.
  - b. Pour this into a 50 mL Erlenmeyer flask (the shape of this flask allows you to swirl the solution to mix it without spilling).
  - c. Add 10 drops of starch indicator solution.
  - d. Set up the 50 mL buret on the the ringstand.

- e. Use a funnel to carefully fill the buret with your iodine titration solution. Tip: the fluid level should not be past the graduated markings on the buret.
  - f. Write down the initial volume of the iodine titration solution in the buret.
  - g. Place the Erlenmeyer flask (containing the vitamin C and starch solutions) under the buret.
  - h. Carefully release the spring clamp of the buret to add iodine solution drop by drop.
  - i. Swirl the flask to mix in the iodine solution after each addition.
  - j. The titration is complete when the iodine creates a blue-back color in the solution that lasts for longer than 20 seconds.
  - k. Record the final volume of the iodine solution in the buret.
  - l. The difference between the initial volume and the final volume is the amount of iodine titration solution needed to oxidize the vitamin C.
  - m. Repeat this step three times. You should get results that agree within about 0.1 mL.
7. Here's how to prepare fresh-squeezed orange for testing.
- a. Use a juicer to squeeze orange juice from two (or more) oranges.
  - b. You need 20 mL of juice per titration, and you should do at least three titrations per storage condition, for a total of 60 mL.
  - c. Filter the orange juice through cheesecloth to remove any pulp and seeds.
8. Titrating an orange juice sample is quite similar to titrating the vitamin C standard. Here are the steps:
- a. Tip: if any of the orange juice samples contain pulp, filter them through clean cheesecloth before doing the titration.
  - b. Use a clean 25 mL graduated cylinder to measure 20 mL of the fresh-squeezed juice.
  - c. Pour this into a 50 mL Erlenmeyer flask (the shape of this flask allows you to swirl the solution to mix it without spilling).
  - d. Add 10 drops of starch indicator solution.
  - e. Set up the 50 mL buret on the the ringstand.
  - f. Fill the buret nearly full with your iodine titration solution.
  - g. Write down the initial volume of the iodine titration solution in the buret.
  - h. Place the Erlenmeyer flask (containing the vitamin C and starch solutions) under the buret.
  - i. Carefully release the spring clamp of the buret to add iodine solution drop by drop.
  - j. Swirl the flask to mix in the iodine solution after each addition.
  - k. The titration is complete when the iodine creates a distinct color change in the juice/starch solution. This color change will be harder to see than with the vitamin C solution, since the juice starts out orange. The color will change from orange to grayish brown when the endpoint is reached. If you continue to add iodine, the color will darken further. You want to note the volume of iodine added when the color *first* changes.
  - l. Record the final volume of the iodine solution in the buret.
  - m. The difference between the initial volume and the final volume is the amount of iodine titration solution needed to oxidize the vitamin C.
  - n. Repeat this step three times. You should get results that agree within about 0.1 mL.
9. For each juice (fresh, premium, or from-concentrate), calculate the average amount of iodine needed to titrate a 20 mL sample.
10. You can calculate the amount vitamin C in your samples by setting up a proportion. Here's an example (with made-up numbers) to show you how:
- a. Let's say that it took an average of 8.5 mL of iodine solution to titrate 20 mL of 1 mg/mL vitamin C standard solution, which means 20 mg vitamin C total.
  - b. Let's also say it takes an average of 6.8 mL of iodine solution to titrate a 20 mL test sample of orange juice.
  - c. We'll call the amount of vitamin C in the orange juice sample  $x$ . You can find what  $x$  is with the following equation:
- $$x/6.8 \text{ mL} = 20 \text{ mg}/8.5 \text{ mL} = 16.0 \text{ mg}$$
11. Did one type of orange juice have more vitamin C than the others? Can you explain your results?

## Variations

- Measure the amount of vitamin C in fresh fruits and vegetables. Which ones have the highest vitamin C content? Which ones have the lowest? Puree a 100 g sample of the fruit or vegetable, and strain it through cheesecloth with about 50 mL of distilled water. Add distilled water to bring the total volume up to 100 mL. From your measurement, you can figure out how much vitamin C there is per 100 g of the fruit or vegetable you sampled. You can use typical weights of the fruit or vegetable to calculate how much total vitamin C there is in each fruit or vegetable you test.
- Measure the vitamin C content in fruit juices. Do the amounts correspond to what is on the label? Design an experiment to find out.
- Does the amount of vitamin C in fruit juice decrease during refrigerated storage? Does the type of container (e.g., glass bottle, or paperboard carton) matter? Design an experiment to find out.
- For an experiment on how storage affects the amount of vitamin C in oranges, see the Science Buddies project [Do Oranges Lose or Gain Vitamin C After Being Picked?](#)
- For more science project ideas in this area of science, see [Chemistry Project Ideas](#).

## Credits

Andrew Olson, Ph.D., Science Buddies

## Sources

This project is based on:

- University of Canterbury, date unknown. "Determination of vitamin C Concentration by Titration," Science Outreach Programme, College of Science, University of Canterbury, Christchurch, New Zealand [accessed July 18, 2007] [http://www.outreach.canterbury.ac.nz/chemistry/documents/vitamic\\_iodine.pdf](http://www.outreach.canterbury.ac.nz/chemistry/documents/vitamic_iodine.pdf).

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