  Spectral Analysis 14

Determination of   
Chlorophyll in Olive Oil

Olive oil is made by pressing or extracting the rich oil from the olive fruit. It seems like a simple matter to press the olives and collect the oil, but many oil extraction processes exist for the many different types of olives grown around the world. To complicate things further, there are also various grades of olive oil, and carefully selected groups of officials meet to define and redefine the grading of olive oil. To help make our experiment a more scientific and less political exercise, we will winnow our investigation of olive oil down to a manageable few variables.

After processing, olive oil comes in three common grades: extra virgin, regular, and light. Extra virgin olive oil is considered the highest quality. It is the first pressing from freshly prepared olives. It has a greenish-yellow tint and a distinctively fruity aroma because of the high levels of volatile materials extracted from the fruit. Regular olive oil is collected with the help of a warm water slurry to increase yield, squeezing every last drop of oil out of the olives. It is pale yellow in color, with a slight aroma, because it contains fewer volatile compounds. Light olive oil is very light in color and has virtually no aroma because it has been processed under pressure. This removes most of the chlorophyll and volatile compounds. Light olive oil is commonly used for frying because it does not affect the taste of fried foods, and it is relatively inexpensive.

The visible light absorbance spectrum of chlorophyll gives interesting results. The chemistry of chlorophyll (some references site four types: a, b, c, and d) creates absorbance peaks in the   
400–500 nm range and in the 600–700 nm range. The combination of visible light that is not absorbed appears green to the human eye, but different sources of chlorophylls will have different ratios of these peaks, which create various shades of green. The ability of chlorophyll to soak up light energy across a wide swath of the visible range helps power photosynthesis at optimum efficiency in plants.

In this experiment, you will have two primary goals. First, you will analyze the various grades of olive oil to determine the absorbance peaks that are present and the relative amount of chlorophyll found in each grade. You will use a spectrometer to measure the absorbance of the olive oil samples over the visible light spectrum. You will then test an unknown sample of olive oil and grade it as extra virgin, regular, or light.

OBJECTIVES

* Measure and analyze the visible light absorbance spectra of three standard olive oils: extra virgin, regular, and light.
* Measure the absorbance spectrum of an “unknown” olive oil sample.
* Identify the unknown olive oil as one of the three standard types.

MATERIALS

Chromebook, computer, or mobile device

Vernier Spectral Analysis app

spectrometer

samples of three olive oil standards: extra virgin, regular and light

olive oil of unknown grade

five cuvettes and lids

plastic Beral pipets

distilled water

isopropyl alcohol

PROCEDURE

1. Obtain and wear goggles.
2. Obtain small volumes of the three standard and one unknown olive oils. Transfer enough of one olive oil sample to fill a cuvette 3/4 full. Place a lid on the cuvette and mark the lid. Prepare all of your samples in this way so that you have four cuvettes of olive oil with labeled lids.

Part I  Comparing Three Grades Of Olive Oil and Identifying an Unknown

For Part I of this experiment, you will calibrate the spectrometer with distilled water. Your goals are: (1) to compare the absorbance spectra of the different grades of olive oil; and (2) to identify the grade of an unknown sample of olive oil.

1. Launch Spectral Analysis. Connect the spectrometer to your Chromebook, computer, or mobile device. Select Absorbance vs. Wavelength.
2. To calibrate the spectrometer, place the blank cuvette in the spectrophotometer and select Finish Calibration. Note: If necessary, wait for the spectrophotometer to warm up before selecting Finish Calibration.
3. Conduct a full spectrum analysis of an olive oil sample.
   1. Remove the blank from the spectrometer.
   2. Place one of the olive oil samples in the spectrometer. Note: Make sure to align the cuvette so that the clear sides are facing the light source of the spectrometer.
   3. Start data collection. A full spectrum graph of the olive oil will be displayed.
   4. Stop data collection.
   5. Review the graph to identify the peak absorbance values.
4. Repeat Step 5 with the remaining olive oil standard samples. Note: The previous data set is automatically saved.
5. Repeat Step 5 with the unknown.
6. Examine the plots of the olive oil samples. Before continuing with data collection, answer the Part I Data Analysis questions and save your experiment file as directed.
7. Rinse and clean the cuvettes and other oil-bearing containers with isopropyl alcohol.

Part II  Comparing the Chlorophyll Concentration of Regular and Extra Virgin Olive Oil

In Part II, you will use the light grade of olive oil to calibrate the spectrometer and presume that light olive oil contains no chlorophyll. Next, you will compare the chlorophyll content of the regular grade with the extra virgin grade.

1. Calibrate the spectrometer using light olive oil.
   1. Click or tap File, , and choose New Experiment.



* 1. Select Absorbance vs. Wavelength.
  2. Prepare a blank by filling an empty cuvette 3/4 full with light olive oil.
  3. Click or tap Settings, , and choose Calibrate.



* 1. Place the light olive oil blank cuvette in the spectrophotometer and select Finish Calibration. Note: If necessary, wait for the spectrophotometer to warm up before selecting Finish Calibration.

1. Measure the absorbance spectrum of regular and extra virgin olive oil.
   1. Remove the cuvette of light olive oil from the spectrometer and replace it with the cuvette of regular olive oil.
   2. Start data collection. A full spectrum graph of the regular olive oil will be displayed. Note the slight difference in the plot as a result of using the light olive oil as the calibration blank.
   3. Stop data collection.
   4. Repeat data collection to measure the absorbance spectrum of the extra virgin grade. Note: The previous data set is automatically saved.
2. Answer the Part II Data Analysis questions, and save your experiment file as directed.

DATA ANALYSIS

Part I  Comparing Three Grades Of Olive Oil and Identifying an Unknown

1. Describe the graph of each of the standard olive oil solutions. Emphasize the differences between each grade of olive oil, identifying the absorbance peaks and other distinguishing features.
2. Compare the absorbance spectra of the three grades of olive oil with the sample graph in Figure 1. What evidence is there that regular and extra virgin olive oil contain chlorophyll while the light grade of olive oil does not?

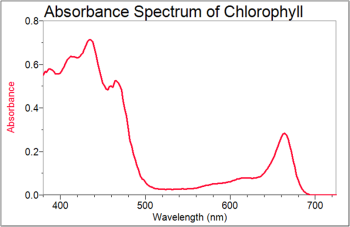


Figure 1

1. Identify your unknown olive oil as extra virgin, regular, or light. Explain your choice.

Part II  Comparing the Chlorophyll Concentration of Regular and Extra Virgin Olive Oil

1. Which grade of olive oil, regular or extra virgin, contains the greater amount of chlorophyll? Use your absorbance spectrum graphs to speculate about how much more chlorophyll one grade contains compared to the other.

Extensions

1. Chlorophyll is a fluorescent molecule. Fluorescent molecules can absorb light of one wavelength and then reemit light at a new and longer wavelength of light. As you have seen in this exercise, chlorophyll absorbs light in the violet and blue regions of the spectra. If you were to shine a violet or blue light through a sample of extra virgin olive oil, you would see the oil turn red in color. The intensity of the red color is an indication of how much chlorophyll is in the olive oil. The “Long-Wave UV Pen Light” from Bio-Rad Laboratories, Inc. (Catalog # 166-0530EDU) can be used for this purpose. Shine the light from the Long‑Wave UV Pen Light through a cuvette containing extra virgin olive oil. Does the sample that is hit by the light turn red in color? Repeat this test for regular olive oil, light olive oil, and your unknown. Could you use this method to determine if a sample of olive oil is really extra virgin olive oil? Could you use this method to determine the grade of any sample of olive oil?
2. Fluorescence spectroscopy is a method that is used to quantify fluorescent compounds in solution. In fluorescence spectroscopy, a sample can be “excited” with a chosen wavelength of light and the resulting light that is emitted from the sample can be measured and quantified. Follow the directions below to measure the fluorescence of all of your olive oil samples.
   1. Launch Spectral Analysis. Connect the spectrometer to your Chromebook, computer, or mobile device. Select Fluorescence vs. Wavelength.
   2. Place the cuvette containing the extra virgin olive oil into the cuvette slot of the spectrometer.
   3. Change the excitation wavelength to 405 nm in the Collection Settings menu.
   4. Change Integration Time to 150 ms in the Collection Settings menu.
   5. Start data collection. A full spectrum graph of the fluorescence of the oil will be displayed. Note that one area of the graph contains a peak at approximately 675 nm. This peak is from chlorophyll. Stop data collection.
   6. The height of the peak should be between 0.6 and 1.0. If necessary, adjust the integration time to increase or decrease the size of the fluorescent peak and repeat the data collection.
   7. Once you have a nice peak, collect full spectrum graphs from the remaining olive oil samples. Do not adjust the sample time. Note: The previous data set is automatically saved.
   8. Compare the fluorescent spectra of the three grades of olive oil. The peak that is visible at approximately 675 nm is from chlorophyll. Which sample has the largest peak in this region?
   9. Using the fluorescence from the known olive oil samples as your standards, determine the quality of your unknown olive oil sample.
   10. Compare your results using fluorescent spectroscopy to your results using traditional spectroscopy. Is one method better than the other? If so, please explain why.
   11. Print or save your experiment file as directed