Determination of Chlorophyll in Olive Oil

Olive oil is made by pressing/extracting the rich oil from the olive fruit by various methods. There are various grades of olive oil; three common grades are: 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 chlorophyll and other volatile materials extracted from the fruit. Regular olive oil is collected with the help of a warm water slurry to increase yield. 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 no aroma because it has been processed under pressure to remove 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 absorbance spectrum, in the visible light range, of chlorophyll gives interesting results. The chemistry of chlorophyll (some references site four types: a, b, c, and d) creates three yellow absorbance peaks at 413, 454, and 482 nm, and two blue absorbance peaks at 631 and 669 nm. The combination of these wavelengths is green to the human eye, but different sources of chlorophylls will have different ratios of these peaks, which create various shades of green. Thus, in the world of chlorophyll, all greens are not the same.

First, you will analyze a sample of the three grades of olive oil to determine the absorbance peaks that are present. You will use a Vernier Spectrometer (V-SPEC) to measure the absorbance of the olive oil samples over the visible-near infrared (NIR) light spectrum (380 – 950 nm). You will then test an unknown sample of olive oil and grade it as extra virgin, regular, or light.

OBJECTIVES

In this experiment, you will

- 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

Vernier Spectrometer
computer
one cuvette
plastic Beral pipets
samples of three olive oil standards: extra virgin, regular and light
distilled water
isopropyl alcohol

PROCEDURE

1. Obtain and wear goggles.
2. Use a USB cable to connect a Vernier Spectrometer to a computer.
3. Start the Logger Pro program on your computer.
4. Obtain small volumes of the three standard olive oils to be tested.
5. To set up the spectrometer, open the Experiment menu and select Connect Interface → Spectrometer → Scan for Spectrometers.
6. Calibrate the spectrometer.
   a. Prepare a *blank* by filling an empty cuvette ¾ full of distilled water.
   b. Open the Experiment menu and select Calibrate → (Spectrometer). The following message appears in the Calibrate dialog box: "Waiting … seconds for the device to warm up." After 90 seconds, the message changes to: "Warmup complete."
   c. Place the blank in the cuvette holder of the spectrometer. Align the cuvette so that the clear sides are facing the light source of the spectrometer. Click "Finish Calibration", and then click OK.
7. Conduct a full spectrum analysis of an olive oil sample.
   a. Empty the blank cuvette and rinse it twice with small amounts of extra virgin olive oil. Fill the cuvette ¾ full with the olive oil and place it in the spectrometer.
   b. Click ▶ Collect. A full spectrum graph of the olive oil will be displayed. Review the graph to identify the peak absorbance values. Click ■ Stop to complete the analysis.
   c. To save your data, select Store Latest Run from the Experiment menu.
8. Repeat Step 7 with the remaining olive oil standard samples.
9. Obtain an unknown sample of olive oil. Repeat Step 7 with the unknown.
10. To save your experiment file, select Save As… from the File menu. (Optional) Print a copy of each graph for your lab report.
11. Select Exit from the File menu to close down Logger *Pro*.
12. Rinse and clean the cuvettes and other oil-bearing containers with isopropyl alcohol.

DATA ANALYSIS

1. Describe the graph of each of the standard olive oil solutions. Emphasize the differences between each grade of olive oil.

2. Identify your unknown olive oil as extra virgin, regular, or light.

3. The introduction of this experiment identified six visible light wavelengths of peak absorbance for chlorophyll. Describe each of the standards, as well as your unknown, in terms of these expected peaks.

Teacher Information

1. There can be a wide variety of types and grades of olive oil available in a grocery store. The International Olive Oil Council defines several grades of olive oil, but the United States Department of Agriculture has its own grading standards. Any three samples that appear, to the naked eye, to have different greenish tints will provide suitable results.

2. The cuvette should be ~ ¾ full to get good absorbance measurements and allow enough room to seal the cuvette with a plastic cap. It is important to seal the samples to avoid time consuming clean up of any spilled oils.

3. Because this lab is qualitative in nature, and olive oil can be difficult to clean from a cuvette, students can prepare their samples in different cuvettes and still achieve good results.

4. An interesting extension for this lab is measuring the absorbance spectrum of chlorophyll extracting from fresh green leaves and comparing it to the absorbance spectra from olive oil samples. There are several methods of extraction that all work well. A simple method is to tear or grind a few leaves and soak them in ethanol or isopropanol for at least thirty minutes.

5. We included a few questions in the Data Analysis section of the student version as a generic starting point. Please feel free to edit or replace these questions as best fits the needs of your experiment.

6. As a good way to become familiar with this experiment, you should plan to keep a set of sample data as well as develop an answer key. It is our experience that data can vary, based on many factors, and the sample data that we have collected in testing this experiment may not be representative of your students' results.

