

Application Note

KEYWORDS

- Honey
- Adulteration
- Food fraud

TECHNIQUES

- Fluorescence
- Laser induced fluorescence

APPLICATIONS

- Counterfeit detection
- Flavonoids measurement

Spectral Detection of Honey Adulteration

Laser Fluorescence Method Hits the Sweet Spot



Background

With its natural sweetness, long history and popularity as an "artisanal" food, honey is among the world's most popular flavors. For centuries, honey has sweetened our foods and beverages and served a wide range of medicinal purposes.

The diversity in flavor, color and aroma of honey comes from the variety of flowers where bees find their nectar and the environment in which the flowers grow. Different flowers and environments produce unique nectars with varying compositions. The result is a nearly endless spread of honey varieties.

As a premium-priced, all-natural product, honey is often adulterated with substances such as corn syrup, molasses, starch and water to fool the consumer with a finished product that is less than 100% pure. By some accounts, honey ranks in the top five targets of food fraud, right after olive oil and milk. Detecting adulterated honey is a challenge due to natural

variations in composition arising from nectar differences. Additional variability is added via processing and storage conditions.

Fluorescence Detection for Discriminating Honey Samples

Fluorescence spectroscopy is an appealing method for distinguishing pure from adulterated honey, as various honey constituents have identifiable fluorescence response (1, 2). These spectral differences could be used for the rapid screening of honey to enable detection of counterfeit products.

Fluorescence measurements are ideal for a rapid screening of honey samples because they are nondestructive and simple to perform. Little to no sample preparation is necessary and testing does not require complicated or expensive instrumentation or highly trained personnel. Also, fluorescence measurements of honey have the potential for use as a quality assurance tool, by detecting degradation features in honey associated with the use of excessive heat for liquefaction or pasteurization (2).

Case Study: Laser Induced Fluorescence of Honey

Researchers at the Agricultural Engineering Research Institute (AENRI) in Cairo, Egypt, used an Ocean Insight modular spectrometer for fluorescence detection with excitation from various laser wavelengths to make laser induced fluorescence (LIF) measurements of honey. Their goals were to characterize the fluorescence spectra for pure, adulterated, heated and stored honey and to devel-

op a method for the simple, rapid and nondestructive detection of honey quality and adulterated honey (2). The researchers observed many spectral differences among the samples that enabled them to distinguish pure honey from adulterated honey. For example, molasses at 1% concentration exhibits a slight shoulder in the spectral curve. Also, the team characterized honey freshness using LIF measurements.

Additional Experimentation

Inspired by the laser fluorescence results, we made similar measurements using an Ocean Insight back-thinned CCD-array spectrometer and a 365 nm LED for fluorescence excitation.

Fluorescence spectra were measured for several types of honey purchased from a local grocery store. Honey varieties included clover honey, golden blossom honey, orange blossom honey and organic honey, ranging in price from about \$0.45/oz. to nearly \$1.00/oz. All samples were produced in the U.S. except for the organic brand, which came from Brazil.

Undiluted honey was pipetted into a disposable cuvette and placed into a sample holder with the excitation and emission fibers arranged at 90 degrees. The fluorescence spectra measured for the honey samples (measurement time was kept constant for all samples) are shown in **Figure 1**. Differences in fluorescence intensity and subtle differences in spectral shape are observed for all samples.

The broad fluorescence peak observed between 400-700 nm in each spectrum results from the presence of flavonoids (antioxidant compounds)



in the sample. Variations in the shape of these fluorescence spectra are primarily attributed to differences in the flavonoid composition of the nectar used to make the honey. Note that the small peak at 365 nm is not fluorescence from the honey but excitation energy that is scattered into the spectrometer by the undiluted, optically dense honey samples.

The flavonoids that dominate the fluorescence spectra for honey (Figure 1) are polyphenols. These plant metabolites determine the color, aroma and flavor of the honey, and provide antioxidant and other health benefits. The unique fluorescence spectrum for each honey sample illustrates the power and sensitivity of fluorescence spectroscopy for characterizing honey.

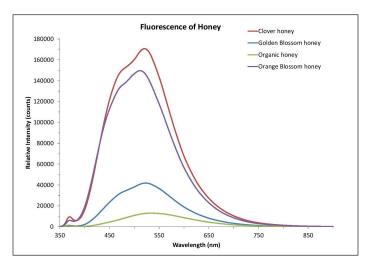


Figure 1: Fluorescence of pure honey with 365 nm excitation. The spectra are dominated by fluorescence response due to the flavonoids present in the honey.

Conclusions

The measurements we conducted focused on the fluorescence of a small set of pure honey samples using a single excitation wavelength. Additional measurements (like those done in Egypt and at other research labs around the globe) could easily be done using the vast array of modular spectroscopy components available. Measurements could be expanded to use a range of LEDs for fluorescence excitation to find the optimal excitation wavelength for the detection of honey adulterants. Also, Ocean Insight Vis-NIR spectrometers and classification models have been used for honey discrimination.

In either case, through the use of modular spectroscopy components, measurements could be taken out of the laboratory setting to test honey quality during bottling or at the point of sale to authenticate that the honey is 100% pure.

References

- **1.** Ruoff, Karoui, et al. "Authentication of the Botanical Origin of Honey by Front-Face Fluorescence Spectroscopy. A Preliminary Study," Journal of Agriculture and Food Chemistry, April 2005.
- **2.** El-Bialee, Rania, et al. "Discrimination of Honey Adulteration Using Laser Technique," Australian Journal of Basic and Applied Sciences, 7(11) Sept 2013, Pages: 132-138



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