Raman spectroscopy for edible oil analysis

Katie Cottingham

- **Many edible, plant-based oils can reduce cholesterol levels and lower heart disease risk.**
- **On grocery store shelves, edible oils are not always what they seem—olive and avocado are oils that are most likely to be mixed with other oils or sold as a higher quality than what is true.**
- **Some countries mandate compliance with olive oil standards, but, except for California, compliance is voluntary in the US.**
- **Raman spectroscopy and other optical methods in which users do not need to know what they are looking for beforehand hold promise as potential screening methods to flag edible oils of dubious quality and purity for further testing.**

There is nothing like munching on a piece of crusty ciabatta dredged through a peppery, buttery olive oil at the start of a meal. Likewise, a quick drizzle of amber-colored sesame oil can liven up a rice or vegetable dish.

After many years of being under scrutiny, oils have become popular again. Some of the most popular plant-based oils, such as olive, soybean, and canola, are high in unsaturated fat and low in saturated fat, which means that these oils can help reduce cholesterol levels and lower the risk of developing heart disease. In addition, edible oils can contain vitamins and antioxidants that promote good health.

But are you really getting what you think you are buying at the grocery store? Intentional adulteration, as well as unintentional trace contamination, are important concerns for economic, health, and sensory reasons, and news reports suggest that olive oil is the most likely oil to be doctored.

Internationally, several entities regulate and set standards for olive oil quality and purity, and experts are now starting to consider standards for other edible oils, such as avocado. The official methods for assessing olive oil products can be labor-intensive, setting the stage for a cadre of researchers developing fast, easy-to-use techniques. Raman spectroscopy and other optical methods in which users do not need to know what they are looking for beforehand are commonly used, but how do they measure up, and could they be accepted as official methods in the future?

DECEPTIVE OILS

The exact number of adulterated edible oils on store shelves is unclear, and estimates vary. Online searches for data on “fake” olive oils frequently turn up figures between 69% and 80%, and they often cite a 2010 report issued by Selina Wang’s team when she was at the University of California, Davis Olive Center (https://tinyurl.com/2yb68bep). She is now associate professor and department vice-chair of cooperative extension in small scale fruit and vegetable processing at UC Davis. However, Wang says that the results of the analysis, which was not published in

a scientific, peer-reviewed journal, are often overblown and misinterpreted.

The analysis was the first large scientific examination of olive oil quality with real domestic and imported samples purchased from stores in California. "We did not say any of the olive oil in that study was fake," says Wang. "The biggest issue we found was that 69% of the olive oil samples, especially the imported ones, did not meet the US Department of Agriculture (USDA) quality standards to be labelled 'extra virgin,' which includes both chemical parameters and sensory analyses."

But mixing an expensive oil with cheaper ones, or even completely substituting one oil for another does happen. Francesca Venturini, professor at the Zurich University of Applied Sciences (ZHAW), recalls that every few years, a report shows up in the news saying that authorities have discovered fraudulent oils. In fact, in a later study of private label avocado oils sold as a retailer or grocery store's brand, Wang found that 70% were not pure, and three of the 36 samples she tested were actually 100% soybean oil (https://doi.org/10.1016/j.foodcont.2023.109837). "We could tell that these products were unequivocally adulterated because soybean oil looks very different from avocado oil based on the fatty acid profile," she explains.

Although dilution with some types of vegetable oils probably would not be harmful, the addition of oils from foods that some people are allergic to, such as peanuts or hazelnuts, could lead to serious health complications. In addition, consumers buying a diluted extra-virgin olive oil will not get the health benefits they were counting on, explains Venturini.

According to experts, instances of faked oils are only expected to increase. "Adulteration is likely to occur because olive and its oil supply has been unsteady and low, while the demand is high," says Wang. "This is an environment that inspires economically motivated adulteration." Recent extreme droughts in the Mediterranean region, the area responsible for most of the extra virgin olive oil supply, have greatly reduced production, while demand has increased from consumers who desire a more healthful lifestyle (https://tinyurl.com/5xxcz48n).

STANDARDS AND REGULATIONS

Olive oil standards and regulations are almost as complex as the oil itself. Many players are involved, though few entities have the power to enforce the standards they have devised.

Globally, two organizations develop standards for categorizing olive oils. The International Olive Council (IOC) is an intergovernmental organization of member countries and entities, such as the EU, that produce olives or olive-based products (https://www.internationaloliveoil.org/). The Codex Alimentarius Commission (CAC) has a broader purview, setting standards for many foods (https://tinyurl.com/8vvbbhwn). Both organizations have now adopted the same standards and testing methods, noting acceptable ranges for the concentration of various molecules and specific chemical parameters. They also state the ideal organoleptic, or sensory, features for trained tasters to detect. Although compliance with these standards is voluntary, many countries use them for governmental regulation. For example, the EU mandates that member states perform checks on olive oil to verify chemical and sensory characteristics, as well as the country of origin (https://doi.org/10.3390%2Fnu12072150).

In the US, USDA and the Food and Drug Administration (FDA) each have some oversight of edible oils. USDA has voluntary guidelines for what can be called extra-virgin, virgin, or regular olive oil (https://tinyurl.com/3cy73x4m). In addition, manufacturers can send samples to the agency for chemical and organoleptic analyses (https://tinyurl.com/mwm3nddn). FDA can inspect food imports, such as edible oils, when they arrive at ports, detaining those foods that are not in compliance with US requirements (https://tinyurl.com/uv6c6ubn). However, although FDA scientists develop and publish new methods for olive oil authentication in journals, they are not used to test products coming into US ports (https://tinyurl.com/3kzwh5kw). This situation could soon change. The House Agricultural Committee issued a report in 2016 calling for FDA to start testing imported olive oils, and in 2019, the American Olive Oil Producers Association—a US olive oil trade organization—and an olive oil company, Deoleo, submitted a citizen petition for FDA to adopt mandatory and enforceable standards. The North American Olive Oil Association joined the petition in 2022.

Another level of complexity arises for growers in California, which was the first state to adopt mandatory standards for olive oils produced within its borders in 2014. "It was a clean slate because it was a new standard that only applied to producers of greater than 5,000 gallons in California, and we were able to include chemical parameters, such as pyropheophytins and diacylglycerol, that were not part of the IOC or Codex standards," says Wang, whose lab provided the data to support the standards. In addition, Wang helped develop the rules.

RAMAN AT THE READY

The official methods stated in the standards can be time consuming. "All of these methods require different instruments and a lot of sample handling and preparation at a chemistry laboratory," explains Venturini.

For example, one type of gold-standard chemical method for assessing parameters, such as fatty acid content and sterols, is gas chromatography—which requires trained personnel, large volumes of reagents, and many hours to prepare and run a sample. Analyzing sterols with the standard chromatography-based method can take as long as three days, says Wang.

But an inspector at a port staring at hundreds of cases of olive oil does not have three days and a fully equipped laboratory to determine if that cargo is adulterated, and that is where optical methods like Raman spectroscopy can shine.

"Raman spectroscopy has an advantage in terms of speed," says Karen Esmonde-White, product manager at Endress+Hauser Optical Analysis in Ann Arbor, Michigan. "I

Compounds and characteristics frequently mentioned in standards for olive oil quality and purity testing:

- **Free fatty acids** (often referred to as "acidity")
  Degradation and hydrolysis can break down the oil's triacylglycerols into free fatty acids. These compounds can also be present if rotten or damaged olives were used.
- **Peroxides**
  High levels of peroxides indicate oxidation.
- **UV light absorbance**
  Certain oxidized compounds are measured by UV absorbance, and this is an indicator of high heat exposure and possible refining.
- **Fatty acid profiles**
  The identities of the fatty acids in the oil can distinguish between olive oils and some seed oils.
- **Sterols**
  Sterols differ depending on the fruit, seed, or nut. The sterol composition indicates the purity of the oil.
- **Stigmastadiene**
  High levels of stigmastadiene indicate the presence of refined oil because this compound is formed when sterols are heated to high temperatures.

- **Pyropheophytins (PPP)**
  PPPs are thermal breakdown products of chlorophyll, indicating possible adulteration with heat-processed, or refined oil.
- **Diacylglycerol (DAG)**
  DAG measures the ratio of the 1,2 form of DAG to its 1,3 form, which can occur as oil ages or is stored at warm temperatures.

Figure 1. A handheld Raman instrument called the MIRA (from Metrohm) can perform edible oil identification. Source: Metrohm

have taken some measurements in one second, and because it is an optical method, you do not need to spend time preparing the samples." Because reagents are not needed, Raman is a "green" method, Esmonde-White points out. She explains that in the long run, avoiding reagents and consumables can also help offset the somewhat higher cost of Raman instrumentation versus a chromatography set-up.

Browsing the literature yields many papers comparing Raman and Fourier transform-infrared (FT-IR) spectroscopy for edible oil analyses. Both methods are highly specific and yield spectra representing chemical bonds. Because they are untargeted, a user does not need to know beforehand what they are looking for. Analyzing the data is tricky for either method, and it requires chemometrics—models and statistical approaches.

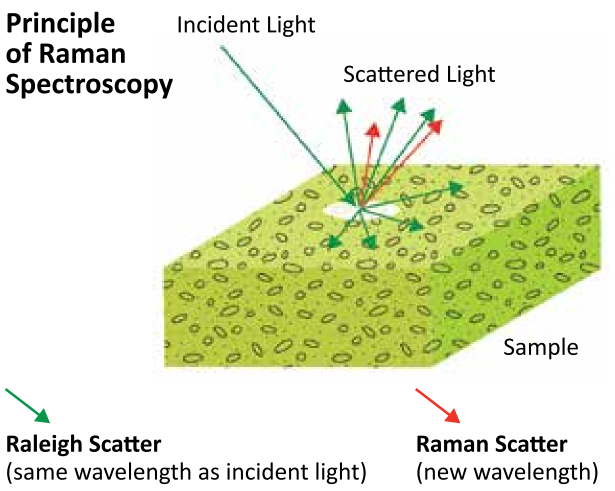
But whereas Raman measures scattered light (see sidebar), FTIR looks at the wavelengths of infrared light absorbed by molecules. "Raman and FTIR are complementary—not everything that you can see with FTIR can be seen with Raman and vice versa," says Venturini. "It depends on the molecules that you are detecting, but for oils, both are very viable, and the advantage they have over other techniques is their specificity."

Raman has a few advantages over FTIR. "Raman works in the visible to near-infrared wavelengths, so you can use more readily available optics," says Esmonde-White. Adam Hopkins, spectroscopy product manager at Metrohm USA in Riverview, Florida, notes sample handling differences. For FTIR, a liquid sample is squished between two plates, and the device has to be cleaned between measurements. "It turns out that people do not do a very good job with that cleaning process," he says. With Raman, however, a user can measure a few drops in a vial, which can be thrown away, without worrying about sample carry-over. And that is right—Raman can even be performed through glass or plastic, but FTIR cannot.

While Raman does a good job of detecting many important molecules involved in edible oil authentication, it lags a bit when it comes to detecting trace contaminants. However, there are ways around that with the recent development of several methods that boost signals, such as surface-enhanced Raman spectroscopy (SERS). In a SERS protocol, gold or silver nanoparticles that are "generally specific" for, say, a certain class of pesticide, are added to a small aliquot of sample, which is shaken, explains Hopkins. Then, the nanoparticles are extracted and analyzed. Venturini has used a different boosting strategy called resonant Raman spectroscopy (RRS) to analyze very small levels of carotenoids, an indication of olive oil freshness (https://doi.org/10.3390/s23177621). With RRS, she used a laser with an energy that corresponded to the absorption of the carotenoids, so that it specifically enhanced only that signal in the spectrum.

THE ROLE FOR RAMAN

In the end, will Raman or other spectroscopy methods ever replace the current official standard methods? "The regulations say you have to do this type of chemical analysis, so you will not get away from that," says Venturini. Wang agrees, saying it is a daunting challenge to propose a brand-new approach to replace standards that were developed through a decades-



Good vibrations: How Raman works

Raman is a non-destructive vibrational spectroscopic method in which a single wavelength laser penetrates through a sample. Some of the laser light interacts with the molecules and is scattered at different wavelengths by the molecules' chemical groups. The intensity of the scattered light is detected, and this forms a characteristic spectrum or fingerprint, which is analyzed against reference spectra for identification or composition analysis. Raman works well on liquids, powders, and solids that have covalent bonds, like pharmaceuticals, organic solvents, and explosives. It does not work well on ionic materials, many metals, and fluorescent materials.

long process with discussion and debate from officials representing many different countries and interests.

However, Wang explains that, with a lot more work, Raman could someday become part of an accepted method. "To believe that an untargeted method can be used reliably, we need to analyze a lot of samples, especially if you couple that with machine learning to develop models," she says. "Much of the work in the literature is more of a proof of concept." It is unclear exactly how many samples would be needed, but she says it could be in the thousands to assess the natural variability—such as different cultivars, geographic origins, and climate—versus intentional adulterations, and this would likely require a large collaborative effort.

A more realistically implementable strategy in the short term is to use portable handheld Raman instruments as a first line of defense for screening at ports and for quality control at a producer's facility—not as an official standard method. "We use handheld Raman instruments to give you that rapid verification aspect," says Hopkins. "The first question it can address is which oil is this, and the second question is whether this is extra virgin, premium, or extra light oil." Then, if a sample fails this type of screen, the lot can be tested further with a more sensitive benchtop instrument or a gold-standard, official method.

Aside from buying a Raman instrument, what can a consumer do? Most experts suggest purchasing products from a reputable brand and just buying what you like. Hopkins says, "Buying from trusted sources and looking at labels is a start, and be suspicious of the price—if it is too good to be true, it probably is."

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