

CO₂ Laser Infrared Optothermal Spectroscopy for Quantitative Adulteration Studies in Binary Mixtures of Extra-Virgin Olive Oil

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ABSTRACT: Optothermal window spectroscopy at CO₂ laser infrared wavelengths was used to detect the extent of adulteration of extra-virgin olive oil by sunflower and safflower oils. A good linearity between the strength of optothermal signal and the concentration of each adulterating compound was found. Predicted limits of detection presently attainable by this new method are 6 (w/w) and 4.5% (w/w) for extra-virgin olive oil adulterated with safflower oil and sunflower oil, respectively, and were confirmed experimentally; the corresponding relative errors were 0.3 and 0.18%. Interference effects are comparable to those encountered in other spectroscopic methods at the same wavelength.

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Consumption of olive oil has increased over the past few years, a trend that may be attributed to its characteristic flavor and potential health benefits. Olive oil is obtained from the fruit of the olive tree (*Olea europaea* L.) and may neither be manipulated nor subjected to any treatment not approved by the recognized international standards (1).

Substitution or adulteration of extra-virgin olive oil by a cheaper ingredient not only is a major economic fraud but can also have potential health implications (2,3). The authentication of extra-virgin olive oil is a major analytical challenge but is often time-consuming and laborious (3). Traditional analytical techniques used for characterizing extra-virgin olive oil, such as determination of iodine and saponification values, measurement of density, viscosity, and refractive index, all require some kind of sample pretreatment (1,2).

Modern high-performance liquid chromatography (HPLC) and gas-liquid chromatography (GLC) techniques provide adequate separation and good sensitivity (4), but require substantial know-how to interpret the results. As an example, adulteration of extra-virgin olive oil by canola oil could be

detected down to 7.5% (w/w) (5). Likewise, mass spectrometry in combination with an artificial neural network is capable of detecting 5% (vol/vol) adulteration of extra-virgin olive oil by soy, sunflower, corn, and peanut oil (6).

Spectroscopy, on the other hand, offers the possibility for direct and nondestructive analysis. For example, ultraviolet spectroscopy (at 210 and 315 nm) was successfully used to detect adulteration of extra-virgin olive oil by refined oil at a level as low as 6% (3). Near-infrared spectroscopy was recently utilized to estimate the level [5% (w/w)] of corn oil, sunflower oil, and raw olive residue oil in virgin and extra-virgin olive oils by using principal component analysis techniques (7).

Although mid-infrared spectroscopy (4000–400 cm⁻¹ region) is often employed for studies of oils and fats, its application for detection of adulteration in olive oil rarely has been reported (3). In contrast to the infrared (IR) spectra of other seed oils, the spectrum of olive oil is rather flat in the 900 to 1200 cm⁻¹ spectral region (1,2). Recently performed (8) quantitative analysis of extra-virgin olive oil, adulterated by walnut oil and refined olive oil, by Fourier transform-IR (FT-IR) spectroscopy combined with attenuated total reflection (ATR) yielded a detection limit of 2% (w/w).

In this paper, the optothermal window (OW) method, a spectroscopic technique related to photoacoustic spectroscopy, was used to determine the levels of the adulterants, safflower and sunflower oils, in extra-virgin olive oil.

EXPERIMENTAL PROCEDURES

Samples used in this study were purchased fresh from a local store. A standard series of blends that contained different proportions (analytical balance) of safflower and sunflower oils in extra-virgin oil was prepared. During the initial phase of the research, the percentage of adulterant in the mixture was varied from 0–100% (w/w) in steps of 20%.

Fatty acid profiles of the methyl esters (9) (Table 1) were obtained by GLC with splitless injection (Carlo Erba 4160 GLC; Carlo Erba Strumentazione, Milano, Italy). The separa-

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tion was achieved on a DB-225 (50% cyanopropylphenyl-methyl polysiloxane) column (15 m \times 0.53 mm, film thickness 1.0 μ m; J&W Scientific, Folsom, CA). The linear velocity of the helium carrier gas was 50 cm/s. The initial temperature (150°C) was programmed to 210°C at a heating rate of 2.5°C/min. The injection and detector temperatures were 250 and 275°C, respectively.

Prior to the actual OW experiments, the spectra (900–1150 cm^{-1}) of all adulterated and pure samples were recorded on a Biorad BST-7 FT-IR (Digit Lab. Dev., Cambridge, MA) spectrophotometer (resolution 2 cm^{-1}) with a deuterated triglycine sulfate detector.

The basic principles behind OW spectroscopy and a detailed description of the experimental set-up are given elsewhere (10,11). The OW constituted a 1.5-mm thick ZnSe disk, and the annular ring made of piezoelectric crystal (PZT) was bonded to the rear side of the disk. A liquid sample (\approx 50 μ L) was deposited directly atop the disk, which in turn was exposed to modulated CO_2 laser radiation. Upon the absorption of radiation in the sample, the generated heat diffused into the disk and caused its expansion, which was sensed by a PZT.

The OW cell used in this study was an improved version of a previous design (11). To avoid Fabry–Perot resonances in the ZnSe disk, the latter was provided with an antireflective coating on the side facing the incident laser beam. Additionally, to prevent laser radiation from directly striking the PZT detector, which would give rise to false signals, a reflective gold layer (5 μ m thick) was deposited onto the same surface in such a way as to leave a clear, uncoated, central circular area about 4 mm in diameter.

RESULTS AND DISCUSSION

The compositions of pure extra-virgin olive oil and of adulterated samples were investigated by GLC measurements and are shown in Table 1. The GLC data showed a linear relationship between the extent of adulteration and the contents of oleic and linoleic acids. The peak areas were expressed as percentages of the total areas (response factors were not

used), and a typical error for these measurements was 3% (for a content of 36%, the error was 1.1%).

The FT-IR experiments were performed first to determine the most appropriate excitation wavelengths of the CO_2 laser (emitting between 931–1084 cm^{-1}). Comparison of FT-IR absorption spectra, obtained from adulterated samples and the pure olive, safflower and sunflower oils, revealed only small differences. In the 900 to 1050 cm^{-1} region, olive oil featured a relatively flat absorption as expected (2). The actual OW studies were carried out (at 293 K) at five selected CO_2 laser wavelengths, i.e., 931, 953, 966, 1041, and 1079 cm^{-1} .

Because of its well-known optical and thermal properties, distilled water was used to calibrate the set-up for OW measurements (11). The 953 and 1041 cm^{-1} CO_2 laser lines were used for the calibration; the absorption coefficients β for water at these wavelengths are $7.92 \times 10^4 \text{ m}^{-1}$ and $5.94 \times 10^4 \text{ m}^{-1}$, respectively (12). The optical alignment and the magnitude (typically $<1 \mu\text{V}$) of a background signal obtained from the empty OW window were checked between successive measurements and after completing the cleaning procedure of the cell (with chloroform).

The plot of the OW signals at 953 cm^{-1} , calculated from experimental results obtained from mixtures of known proportions of safflower oil in olive oil, is displayed in Figure 1. To calculate the absorption coefficients, the OW signals were first normalized to the laser power measured behind the OW cell (11).

Each data point in Figure 1 represents an average of 20 successive measurements, performed at 221 Hz with the CO_2 laser tuned to the 953 cm^{-1} line; at this particular wavenumber, the relative error was 0.3%. In general, the error was found to depend on the power stability of the laser at a specific transition, i.e., 0.7% at 931 cm^{-1} , 0.4% at 966 cm^{-1} , and 0.2% at 1079 cm^{-1} . For olive oil adulterated with safflower oil, the limit of detection (LOD), here defined as the ratio of a threefold standard deviation obtained from the blank (olive

TABLE 1
The Fatty Acid Profiles^a (determined by GLC) of FAME of Olive and Safflower Oils and of Their Mixtures^b

Safflower oil								
(% w/w)	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1
0	13	1	2	73	9	1	<0.5	<0.5
20	11	1	2	62	23	1	<0.5	<0.5
40	11	<0.5	2	49	37	<0.5	<0.5	<0.5
60	10	<0.5	2	36	51	<0.5	<0.5	<0.5
80	8	<0.5	2	23	66	<0.5	<0.5	<0.5
100	7	—	2	11	77	<0.5	<0.5	<0.5
Sunflower oil								
	7	<0.5	4	18	70	<0.5	<0.5	<0.5

^aFatty acids reported as percentage of total fatty acids.

^bComposition of sunflower oil included for comparison. GLC, gas-liquid chromatography; FAME, fatty acid methyl esters.

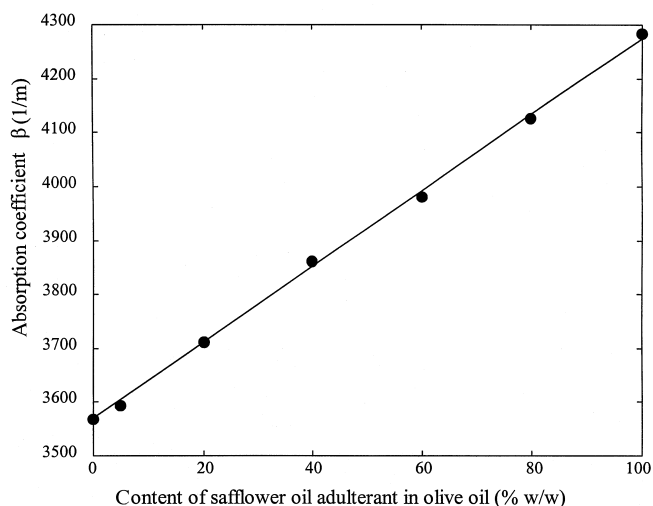


FIG. 1. Experimentally determined absorption coefficient β for olive oil adulterated by safflower oil. The study was performed at the 953 cm^{-1} transition of the CO_2 laser.

oil) and the slope of the calibration curve, was 6% (w/w); the regression coefficient r for data in Figure 1 was 0.9995.

The 8% (w/w) and 6% (w/w) LOD (regression coefficients $r = 0.997$), obtained when studying the above test samples at 931 and 966 cm^{-1} , are ascribed to a poorer stability of the laser at these two lines. At 1079 cm^{-1} , as expected, no difference was observed between the OW signals obtained from safflower and olive oils. On the other hand, differences measured at other lines were found to be due to the optical rather than thermal changes induced in the sample.

Results for olive oil adulterated by sunflower oil, shown in Figure 2, were obtained in the same way as those shown in Figure 1, but with the CO₂ laser tuned to the 1041 cm^{-1} line. The relative error was 0.18%, giving a 4.5% LOD and a regression coefficient r of 0.999.

When deriving the above-stated LOD (Figs. 1 and 2), the concentration range between 0 and 20% (w/w) was originally interpolated. This deficiency was later rectified by preparing extra standards [5% (w/w)] of each adulterant. Both of these samples produced measurable OW signals. As an example, for the extra-virgin olive oil adulterated with 5% (w/w) safflower oil, the absorption coefficient (at 953 cm^{-1}) calculated from the calibration curve (Fig. 1) was 3604 m^{-1} , which is close to that found experimentally, *viz.* 3590 m^{-1} . Similarly, a value of 5059 m^{-1} (at 1041 cm^{-1}) was found compared to the expected 5051 m^{-1} for a specimen that contained 5% sunflower oil in the extra-virgin olive oil.

The OW method used here proved suitable to rapidly detect reasonably small amounts of known adulterants (safflower and sunflower oils) in the extra-virgin olive oil; LOD presently attainable are 5% (w/w). Generally, the obtained LOD are of the same order of magnitude as those attainable for similar adulterants by traditional methods (FT-IR, GLC, HPLC, and mass spectrometry). An enhancement of LOD is anticipated for a more stable, discretely tunable CO₂ laser. The level of background noise for the OW device used in this

study was significantly below that of the OW sensor used previously (11). Manufacturing the thinner window from a material with better optical and thermal properties than currently used ZnSe (e.g., diamond) is expected to lead to an improvement in LOD as well.

In conclusion, it has to be emphasized that the OW study was performed with a discretely tunable CO₂ laser on two binary mixtures, each containing only a single adulterant, the quantities and identities of which were precisely known. Obviously, to identify and quantitate adulterants of unknown nature in a complex mixture, one needs continuously tunable radiation. The presently emerging generation of compact and high-power IR diode lasers will boost both the specificity and sensitivity of the OW method, simply because the excitation wavelengths characterized by the optimal spectral contrast will become available. As for the effect of unwanted spectral interferences at a selected wavelength in the OW approach, this latter is comparable to that normally encountered with any other spectroscopic technique.

One should also bear in mind that the OW device represents a universal detection scheme, i.e., it is not wavelength-limited. This means that one and the same device can be used within a wide range of wavelengths, provided the window remains transparent to the exciting radiation.

When combined with other advantages of the OW technique (practicality, reasonably low cost, suitability for on-line operation, and intrinsic simplicity when loading, cleaning, and operating the cell), the developments mentioned above could eventually make the OW technique a good candidate, not only for rapidly quantifying the presence of unknown adulterants in extra-virgin olive oil but also for many other applications as well.

As to this point, the recent application of OW-based instruments for the analysis of principal components (moisture, fat, protein, carbohydrates) of cheeses at the selected near- and mid-IR wavelengths is noteworthy. Its performance was superior to that of FT-IR-ATR in terms of speed, accuracy and the ease of data analysis (13,14). Work on similar matter is in progress [Wilson, R. (Institute of Food Research, Norwich, England), private communication, 1997].

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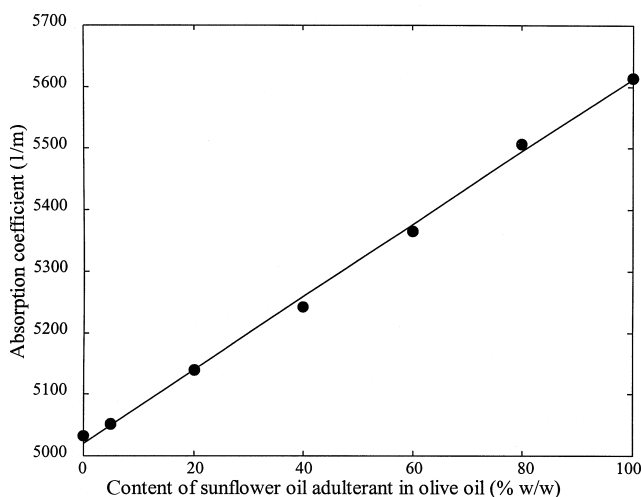


FIG. 2. Experimentally determined absorption coefficient β for olive oil adulterated by sunflower oil. The study was performed at 1041 cm^{-1}

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