A Rapid, Univariate FT-NIR Procedure to Determine Moisture Concentration in Olive Oil

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Abstract: We recently observed that the weak near-infrared (NIR) band near 5260 cm⁻¹ was relatively more intense for extra virgin olive oil (EVOO) than for refined olive oil (ROO). We also observed that its intensity was diminished upon heating and erroneously presumed that it may be attributed to volatile carbonyl components in EVOO. In the present study we demonstrate for the first time that this band is primarily attributed to a water O-H combination band. To accurately determine the intensity of this weak band, observed on a shifted and sloping baseline, we measured the peak-to-peak (p-p) height of its first derivative. An exponential calibration curve for p-p height versus gravimetrically-determined concentration of spiked water was satisfactorily generated. The calibration curve was first evaluated by using independent sets of gravimetrically prepared test samples. Subsequently, it was used to determine the moisture content, a quality parameter, for a limited set of authenticated reference olive oils whose quality and purity were confirmed by official methods. These concentrations, 0.098-0.12% H₂O (w/w) for EVOO, 0.022-0.030% H₂O (w/w) for ROO, and 0.028-0.054% H₂O (w/w) for pomace olive oil (POO), were consistent with those reported in the literature. For 88 commercial products investigated, the moisture levels fell in the range from 0.026% to 0.13% (w/w). The correlation between moisture content and other olive oil quality parameters has been reported in the literature and has yet to be further investigated.

Key words: olive oil, near-infrared spectroscopy, moisture in EVOO, rapid screening

1 Introduction

Virgin olive oil, which is produced by the mechanical pressing of the fruit of the olive tree (Olea europaea L.), is known for its excellent organoleptic properties and nutritional value¹. For many years, extra virgin olive oil (EVOO) has been the target of economically-motivated adulteration (EMA)¹,². In 2016, a U.S. Congressional Committee expressed concern about reports that consistently described the prevalence of imported olive oil sold in the U.S. as adulterated or fraudulently labeled³. Such fraud may potentially have unintended consequences attributed to the mixing of EVOO with other edible oils that could pose a health risk to consumers who are allergic to trace levels of allergenic proteins that may be found in some extraneous oils. Consequently, the U.S. Food and Drug Administration (FDA), which is tasked with protecting the U.S. food supply against deceptive labeling and intentional adulteration, was directed to screen imported olive oils to determine if they are adulterated or misbranded. Therefore, there has been a need for authoritative FDA standards for testing olive oil as well as rigorous analytical methodologies to rapidly and accurately determine olive oil purity and quality.

For decades, near infrared (NIR) spectroscopy has been a common tool used in food analysis and particularly useful for rapid screening. Variation in the intensity of a weak NIR band near 5260 cm⁻¹ was first observed for olive oil by Christy et al.⁴. In recent Fourier transform (FT)-NIR studies in our laboratory⁵-¹⁰, this band was recognized as highly characteristic and potentially useful for discriminating between EVOO and olive oils of lower quality or purity. We reported that it was more intense for EVOO than for lower-grade olive oils and mixtures of EVOO with extraneous edible oils⁵,¹⁰. In these studies⁵,¹⁰, a multivariate partial least squares (PLS) model was developed to estimate the intensity of this weak absorption band near 5260 cm⁻¹ rela-
ative to the adjacent one near 5180 cm$^{-1}$. Further, when an EVOO test portion was placed under vacuum, purged with nitrogen, heated to 50$^\circ$C for 10 min, or mixed with refined edible oils, the intensity of this band was reduced$^{16}$. Therefore, this band appeared to be due to volatile components and we erroneously attributed it to carbonyl vibrations$^{15}$.

In the present study we demonstrate for the first time that, based on spiking experiments, the unique NIR band near 5260 cm$^{-1}$ is attributed to moisture in olive oil. Preliminary data were recently presented at the 2019 InternationalForum Process Analytical Chemistry$^{11}$. The moisture content of olive oil is a quality parameter according to CODEX standard for olive oils and olive pomace oils$^{21}$. Several official methods$^{13-17}$, with varying degrees of accuracy and sensitivity, are available for the determination of moisture in olive oil as well as for other fat and oil matrices. Current analytical techniques, including Karl Fischer titrimetric official method (AOCS Ca 2e-84)$^{13,14}$, other AOCS official methods (AOCS 2a-45, AOCS 2b-38, AOCS 2c-25, AOCS 2d-25)$^{14-17}$, as well as a number of procedures$^{18-24}$ including those based on multivariate chemometric analyses and shared these reference samples with us. Addition-ally, 88 commercial oils labeled as EVOO whose purities were determined with official methods for sterols and triterpene dialcohols (see Results and Discussion) were purchased locally from grocery stores near the College Park, MD area.

2.2 FT-NIR Instrumentation

A Bruker MPA FT-NIR spectrometer equipped with a thermoelectrically cooled InGaAs detector was used in the present study for collecting spectra. All test samples were measured at room temperature with a fiber optic probe in transfection mode at a 2 mm pathlength. The probe was immersed in approximately a 2 mL test portion that was placed in a 10 mL beaker and spectra were collected by adding 32 scans at 8 cm$^{-1}$ resolution and by using the Blackman-Harris 3-term apodization function. The probe was cleaned as follows. Once per day prior to spectral measurement, it was cleaned with a dilute (5% v/v) aqueous solution of a dish liquid detergent, rinsed with water, and carefully dried with Kimwipes. Prior to collecting spectra, for a given new sample to be measured the probe was immersed in a 2 mL portion and wiped clean with Kimwipes. This 2 mL oil portion used for cleaning was discarded and a new 2 mL test portion was used for measuring this sample. For the measurement of each test sample, triplicate spectra were collected and averaged. For commercial olive oil products, soon after purchase and immediately after bottles were opened, all spectra were collected and saved in a database for subsequent data analysis and, if needed, future re-analysis. For quantification, the absorbance spectrum collected over the entire spectral range 4,500-10,000 cm$^{-1}$ was converted to its first derivative (smoothing points = 9) and the p-p height of the first derivative of the band near 5260 cm$^{-1}$ was calculated by adding the absolute values of the maximum and minimum intensity amplitudes of the positive and negative first derivative peaks, respectively.

2.3 Preparation of calibration olive oil samples with increasing gravimetrically-determined concentrations of spiked water

A certified organic EVOO (Lot No. MKBZ8351V) from Sigma Aldrich (St. Louis, MO) (EVOO1), an authentic EVOO (EVOO2, a certified commercial product from Spain) and a certified highly refined olive oil (HROO; Sigma Aldrich) were used for calibration. To expel moisture, approximately 300 mL of an oil was purged with dry UHP...
grade nitrogen for 90 min at a flow rate of at least 10 mL/min measured with a soap film flow meter (Hewlett-Packard, Palo Alto, CA). Subsequently, test portions of this oil were spiked with low concentrations of deionized water, 1804 mΩ (Aqua Solution, Butler, PA). The optimal purge time of 90 min was determined based on the observation that the NIR band near 5260 cm\(^{-1}\) no longer decreased in intensity when purged beyond 90 min for up to 48 hr. Briefly, to each empty 20-mL headspace vial with a screw cap, addition of low concentrations of water via pipette tip and gravimetric evaluation was carried out. This was followed by addition of approximately 10 g of olive oil. Concentrations were typically at 0.01, 0.02, 0.05, 0.07, 0.10, 0.13, 0.15, 0.17, 0.20, 0.22% \(\text{H}_2\text{O (w/w)}\) with weights recorded to a precision of \((\pm 0.0001 \text{ g})\). Each test sample was vortexed (Scientific Industries, Inc., Bohemia, NY) for 1 min at level 10, placed in a shaker (Glass-Col, Large capacity mixer, Terre, Haute, IN) for 15 min (motor speed 80 and with pulser on) at room temperature, allowed to stand for 5 more minutes, and each test portion (2 mL) was removed for measurement by FT-NIR spectroscopy once per day over three days (EVOO1) or two days (EVOO2 and HROO). For all the test samples prepared with these three spiked oils, the p-p heights of the first derivative of the band near 5260 cm\(^{-1}\) observed for the replicate measurements were averaged for a given concentration level and used to generate a calibration curve.

2.4 Independent evaluation test samples

The oils EVOO1, EVOO2 and HROO were also used to prepare independent sets of evaluation test samples (not used for calibration) to determine accuracy. Each oil was used to prepare gravimetrically-determined spiked test samples at three concentration levels, approximately 0.026%, 0.064%, and 0.124% \(\text{H}_2\text{O (w/w)}\) (Table 1) according to the procedure described in Section 2.3.

### Table 1: Evaluation results for the calibration regression function developed using three oils*

| Oil     | Spiked \(\text{H}_2\text{O Concentration (% w/w)}\) | Calculated \(\text{H}_2\text{O Concentration (% w/w)}\) | Accuracy, % |
|---------|---------------------------------|---------------------------------|-------------|
|         | Average (STDEV) \(^b\)           | Average (STDEV) \(^b\)           |             |
| EVOO1   | 0.026                           | 0.031 (±0.002)                   | 116         |
| EVOO1   | 0.064                           | 0.071 (±0.007)                   | 111         |
| EVOO1   | 0.125                           | 0.127 (±0.025)                   | 101         |
| EVOO2   | 0.026                           | 0.029 (±0.003)                   | 110         |
| EVOO2   | 0.064                           | 0.070 (±0.009)                   | 109         |
| EVOO2   | 0.124                           | 0.132 (±0.008)                   | 106         |
| HROO    | 0.026                           | 0.026 (±0.002)                   | 100         |
| HROO    | 0.064                           | 0.068 (±0.007)                   | 106         |
| HROO    | 0.124                           | 0.122 (±0.016)                   | 99          |

* EVOO1 and EVOO2, authentic extra virgin olive oil; HROO, highly refined olive oil.

\(^b\) Standard deviation (STDEV) represents duplicate measurements.

### 3 Results and Discussion

A new, rapid and simple univariate FT-NIR procedure for accurately determining the intensity of the weak water band that occurs on an offset and sloping baseline observed near 5260 cm\(^{-1}\) for olive oils is proposed. It is based on measuring the observed p-p height of the first derivative of this absorption band (Figs. 1 and 2). For weak absorption bands, the measured p-p height of the first derivative is a more accurate metric than the estimated band area of the observed absorption bands. This is because the first derivative transformation of absorption bands successfully eliminated or minimized baseline effects. The FT-NIR measurement (Figs. 1 and 2) of reference materials, i.e., authenticated grades of olive oils whose quality and purity were determined with official methods, is an essential and critical factor for successful method development. Figures 1 and 2a illustrate the difference in the absorption band intensity near 5260 cm\(^{-1}\) for the authenticated EVOO and ROO, while Fig. 2b exhibits the clear difference in their observed first derivative p-p heights. Figure 2c similarly illustrates the difference in the p-p heights for authenticated EVOO and POO. The higher p-p height observed for POO relative to that for ROO is likely due to the fact that, prior to solvent extraction of olive oil, water is the highest pomace constituent and is reportedly found at approximately 50-65%\(^{22}\).

To demonstrate that the moisture content of an olive oil is responsible for the variation in intensity of the band near 5260 cm\(^{-1}\), EVOO1 was purged with \(\text{N}_2\) for 90 min to expel...
moisture and test portions of this oil were subsequently spiked with increasing gravimetrically-determined concentrations of water. Figure 3 illustrates 11 spectra collected for water in EVOO1 in the concentration range of 0.00 to 0.22 (w/w). The corresponding first derivative spectra are shown in the inset. The most intense band near 5260 cm$^{-1}$ was observed for an authentic EVOO, the weakest band near 5260 cm$^{-1}$ was observed for EVOO after purging, and all the bands in between were observed after addition of water.

Fig. 3 Expanded FT-NIR spectral region that exhibits the observed increase in the intensity of the moisture band near 5260 cm$^{-1}$ for an extra virgin olive oil (EVOO1), that had been purged with nitrogen gas to expel moisture, and subsequently spiked with gravimetrically-determined increasing levels of water ranging from 0.01% to 0.22% (w/w). The corresponding first derivative spectra are shown in the inset. The most intense band near 5260 cm$^{-1}$ was observed for an authentic EVOO, the weakest band near 5260 cm$^{-1}$ was observed for EVOO after purging, and all the bands in between were observed after addition of water.

Fig. 4 Observed FT-NIR spectra collected for authenticated oils: six extra virgin olive oils (EVOO) and four refined olive oils (ROO). Spectral differences were observed for the weak, yet highly characteristic, O-H combination moisture band near 5260 cm$^{-1}$.

Fig. 5 Expanded FT-NIR spectral region to illustrate for authenticated oils: (a) absorption bands, (b) the corresponding first derivative bands for six extra virgin olive oils (EVOO) and four refined olive oils (ROO), and (c) first derivative bands for six EVOO and eight pomace olive oils (POO).
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that the concentration range of interest, where water is dissolved in oil, was only up to approximately 0.15\% (w/w).

The solubility of water in oil\(^{26}\) is usually described as consisting of dissolved, emulsified, and free water phases. An exponential curve fit is used in the present study because it adequately describes the physical dissolution of low levels of water in olive oil as the solution reaches a saturation point. An exponential regression curve, \(Y = 0.5667 + (17.87 - 0.5667) \times (1 - \exp(-8.112x))\) obtained with

\[
\text{GraphPad Prism 7.04 (San Diego, CA),}
\]

was successfully generated in the range from 0 to 0.15\% (w/w) for the oils used for calibration, as shown in Fig. 4 for first derivative p-p height (Y) versus added water for gravimetrically-determined concentrations (x) of up to 0.15\% (w/w). As the solubility of water in oil reached saturation, the regression curve leveled-off indicating that a water-in-oil emulsion has progressively formed. In a study that used a narrower concentration range, 0-1000 ppm (0-0.1\% (w/w))\(^{24}\), a linear correlation was reported for FTIR-based calibration of moisture in edible oils. A small intercept was found in the calibration plot (Fig. 4) that may be attributed to potentially interfering minor constituents that absorb near the weak \(\text{O-H water combination NIR band of interest in the purged oil used for gravimetric spiking. The possibility that a residual amount of moisture may also be responsible for the small intercept has yet to be verified with the Karl Fisher}\(^{13}\) official method.

Hilder has shown that the solubility of water in edible oils is independent of the type of oil used when expressed in terms of mole fraction\(^{20}\). This was verified in the present study in an experiment where three different oils (EVOO1, EVOO2, and HROO) were used for generating an average calibration curve, yielding low variability among replicate measurements of three oils (Fig. 4). To further evaluate the accuracy of determination, the calibration curve that we obtained was subsequently evaluated with an independent set of spiked test samples of EVOO1, EVOO2 and HROO, not used for calibration, at three concentration levels (Table 1). Evaluation data indicated good agreement between the gravimetrically-determined added water concentrations and the average calculated water concentrations (Table 1). The average calculated accuracy (106\%) was satisfactory; good agreement between replicate measurements was found with low standard deviations (Table 1). Generally, there was no bias (mean of differences between calculated and gravimetric values), which was found to be low (0.004) for the evaluation test samples. Also, we compared the known spiked water concentrations with the average calculated concentrations at every spiked level prepared from each of the three oils. In this case, satisfactory accuracy was also found. The three spiked concentrations at 0.026\%, 0.064\%, and 0.124\% were calculated as 0.029\% (+ 0.003), 0.070\% (+ 0.002), and 0.127\% (+ 0.005), respectively.

The regression function generated for olive oils in Fig. 4 was subsequently used to determine the moisture content, based on the observed first derivative p-p intensities for all the authenticated and commercial oils investigated. For the limited set of authenticated olive oils investigated the ranges of moisture were as follows: EVOO (0.098-0.12\% \(\text{H}_2\text{O}, \text{w/w}\)), ROO (0.022-0.030\% \(\text{H}_2\text{O}, \text{w/w}\)), and POO (0.028-0.054\% \(\text{H}_2\text{O}, \text{w/w}\)).

Figure 5 illustrates the moisture content calculated for a set of 88 unique commercial brands labeled as EVOO whose levels fell in the range from 0.026\% to 0.13\% (w/w). The moisture content of the limited set of authenticated EVOO ranged from 0.098\% to 0.13\% (w/w). However, the correlation between moisture content and quality has yet
to be investigated. Once a universal moisture threshold is established for EVOO, it would then be possible to rigorously assess olive oil quality.

In the present FT-NIR study, the moisture content determined for EVOO was higher than those obtained for the lower quality POO and ROO and all values were consistent with those reported in the literature. In general moisture concentrations in EVOO versus POO and ROO are reported between 0.1 and 0.2% (w/w) for the former and < 0.05% for the latter. Based on the reference Karl Fisher titration method, moisture concentrations for two ROO were reported to be 210.2 and 211.7 μg/g (0.0210% and 0.0211%, w/w) which align well with the NIR determined values for the authenticated ROO evaluated in this work. Additionally, the water content was predicted for 10 different monocultivar EVOO by time domain reflectometry and partial least square regression (PLSR) and all fell in the range from 714 to 2008 mg water/kg oil (0.0714% to 0.2008%, w/w). Using mid-infrared spectroscopy and PLSR, moisture was also predicted for 47 oils obtained by blending virgin olive oil and ROO to be in the range of 289 to 1402 mg water/kg oil (0.0289% to 0.1402%, w/w). In another study, the EVOO moisture content reported before and after industrial filtration decreased from approximately 0.12-0.14% (w/w) to approximately 0.08 to 0.10% (w/w)

Several reports have correlated moisture concentration to other olive oil quality parameters. EVOO quality is reportedly related to oil composition including its moisture content. This is attributed to the fact that a reduction in moisture content would negatively impact the polar fraction, primarily the phenolic compounds, responsible for the oxidative stability of olive oil particularly during storage. The amount of moisture could reportedly serve as an analytical tool to detect mild deodorization of EVOO; this process has presumably been used to lower the contents of volatile and polar compounds responsible for off flavors. Such practice would decrease the moisture concentration to <700 mg water/kg oil (<0.07%, w/w). The minimum moisture content for establishing EVOO status has yet to be determined.

When the purity, i.e., whether EVOO is adulterated with other edible oils (non-olive oils), of the same set of 88 commercial products labeled EVOO was assessed in our laboratory by applying an International Olive Council (IOC) official method for the determination of the content of sterols and triterpene dialcohols, only three products failed to meet purity criteria. These results obtained with an official method appear to suggest that there is an apparent paucity of adulterated retail EVOO products sold in the U.S.

Information on olive oil quality for commercial products sold in California was reported by the Olive Center, U.C. Davis: 66 out of a total of 90 products (73%) of top-selling, imported brands of EVOO failed the IOC’s standards for EVOO quality as determined by two IOC-accredited sensory panels. This percentage was fairly consistent with those obtained by chemical analyses for quality assessment based on the 1,2-diacylglycerol content (65%) and the pyropheophytins (49%) tests adopted by Australia. The correlation between moisture content and quality parameters will be evaluated and compared with the literature.

4 Conclusions

The weak NIR band near 5260 cm⁻¹ is primarily attributed to low concentrations of moisture (water O-H combination band) in olive oil. A rapid, simple, univariate FT-NIR procedure to quantify moisture in olive oil is proposed. Moisture concentrations were found to fall in the narrow range from 0.098 to 0.12% (w/w) for a limited set of authenticated EVOO and as high as 0.13% (w/w) for 88 retail products labelled as EVOO, which are consistent with literature values. A universal threshold for assessing the minimum EVOO moisture content that would satisfy the requirements imposed by all possible sources of EVOO variability, attributed for instance to geographic origin, climate, soil, crop year, or time of harvest as well as processing conditions, has yet to be determined. This proposed proof-of-concept procedure based on the simple and accurate determination of moisture content, once validated in comparison with an official method and applied to a larger set of representative genuine EVOO products, would potentially be a useful initial screening tool for rapidly identifying and flagging low moisture content for commercial EVOO products. Based on the use of the Karl Fischer official method, a multi-laboratory validation of the proposed FT-NIR procedure for rapidly determining moisture content in olive oil is planned.

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