Application note

Olive Oil Quality Analysis Through UV-Visible Absorption Measurements

Introduction

All food products should be of trustworthy quality and purity, not just for consumer safety but also to ensure product authenticity and the accuracy of product labels. This is especially important for olive oils, of which there are many different types based on quality (e.g. refined, extra virgin, etc.). In some circumstances, high quality oils can be adulterated with other lower quality olive oils or oils from a different source, like canola or soybean.[1,2] Additionally, the overall quality and stability of commercially available olive oil is highly important to avoid undesired organoleptic properties.[2,3] Consequently, rigorous testing of the quality of olive oil products is needed to ensure they are free of undesired adulterants and byproducts. The International Olive Council (IOC) outlines analyses which can be performed to ensure the purity of various forms of olive oil, as well as the associated passing criteria.[4]

One such method involves the use of UV-Visible absorption spectroscopy, a non-destructive analytical technique which monitors the transmission of UV-Visible light through a sample. The ratio between the transmitted light intensity and the initial intensity of the light prior to interacting with the substance is used to calculate absorbance. As the ability to absorb light is based on the electronic structure of the analyte of interest, this technique generates results specific to the analyte. Additionally, through Beer's law, the absorbance is found to be directly proportional to the concentration of an analyte in a medium. As a result, this technique is often used as a quantification method.

UV-Visible absorption spectroscopy has been used to quantify different analytes in olive oil samples, including chlorophyll and carotenoids.[3,5] However, the IOC method specifically uses the absorbance of conjugated dienes/trienes in the products as a means for determining the extent to which a given olive oil has been oxidized.[2,3,6] The oxidation of olive oils can lead to changes in flavor, resulting in a decrease in product quality.[3] In IOC's procedure, the "specific extinction" (K), the extinction coefficient of a 1% w/v oil solution in either cyclohexane or iso-octane, is determined at two wavelengths to assess the amounts of these oxidation byproducts present.[6] Based on the IOC acceptance criteria,[4] these values, in addition to other parameters, are expected to be different for different types of olive oils.

This IOC procedure for determining olive oil purity was demonstrated using a Thermo Scientific™ Evolution™ One Plus UV-Visible Spectrophotometer. Herein, the results for four olive oil samples of varying quality are included as well as the results for a canola oil sample. Using the advanced calculations function available in the Thermo Scientific™ Insight™ Pro Software, calculations were carried out according to IOC's requirements.[6] These results demonstrate the ease with which UV-Visible absorption measurements can assess the extent to which olive oil samples have been oxidized.

Experimental

Experiments were carried out using commercially available oil samples. These samples, as well as the ingredients disclosed on the label, are outlined in Table 1 and depicted in Figure 1. Samples were prepared based on procedures outlined in the IOC method.[6] Briefly, for each oil sample, a ~1 g/100 mL stock solution was made by dissolving the oil in cyclohexane (see Table 1). Diluted oil samples were then made by diluting 0.5 mL of the prepared stock solution with 2.0 mL cyclohexane.

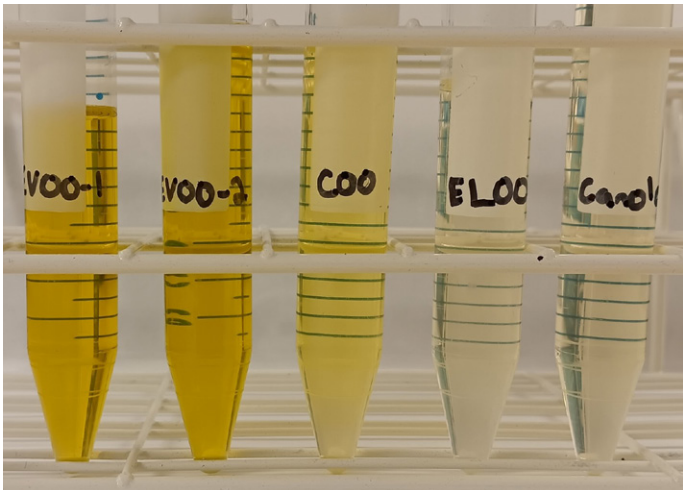


Figure 1. Olive oil samples. From left to right: EVOO-1, EVOO-2, COO, ELOO, Canola Oil.

Labeled Oil Sample	Ingredients on Label	Prepared Stock Concentration (g/100 mL)	Diluted Concentration (g/100 mL)
Extra Virgin Olive Oil – Brand 1 (EVOO-1)	Extra Virgin Olive Oil	1.07	0.215
Extra Virgin Olive Oil – Brand 2 (EVOO-2)	Extra Virgin Olive Oil	1.04	0.208
Classic Olive Oil (COO)	Refined Olive Oil and Virgin Olive Oil	1.00	0.200
Extra Light Olive Oil (ELOO)	Refined Olive Oil and Virgin Olive Oil	1.02	0.204
Canola Oil	Canola Oil	1.00	0.201

Table 1. Description of ingredients of olive oil samples as well as concentration of prepared stock solutions.

UV-Visible absorption measurements were collected of the stock solutions as well as the diluted oil samples using an Evolution One Plus UV-Visible spectrophotometer. All samples were held in a 1.0 cm quartz cuvette. The absorbance at 232 nm, 266 nm, 270 nm and 274 nm was measured using a 1.0 nm bandwidth and 1.0 s integration time.

Results/Discussion

Based on the procedures outlined by IOC, the absorbance at 232 nm and 270 nm needs to be collected when using cyclohexane as a solvent.[6] The former wavelength is used to assess the presence of primary oxidation products, like peroxides, while the latter assesses the amount of secondary oxidation products present (e.g. ketones, trienes, etc.).[2,3] Note that the absorbance measured is dependent on the concentration of the analyte and the path length the light passes through, as per Beer's law (Eqn. 1).

$$A_\lambda = cl\varepsilon_\lambda$$

Equation 1.

This can make comparisons between samples of varying concentration difficult. Consequently, it is often easier to use the extinction coefficient (ε_λ) as a point of comparison as this value is constant and unique to the analyte of interest.

For the analysis of oxidation products in oil, the specific extinction coefficient (K_λ), the extinction coefficient of a 1% oil solution in either iso-octane or cyclohexane, is determined as per Equation 2,

$$K_\lambda = \frac{A_\lambda}{c*l}$$

Equation 2.

where c is the concentration oil solution (units = g/100 mL) and l is the pathlength. In addition, the variance in the specific extinction coefficient (ΔK) is also needed according to IOC's procedure to test for sample purity.[6] This variance, as outlined in Equation 3, is only determined for the absorbance at 270 nm, in cyclohexane, or 268 nm, in iso-octane.

$$\Delta K_\lambda = K_\lambda - \left(\frac{K_{\lambda+4} + K_{\lambda-4}}{2}\right)$$

Equation 3.

Oil Type	*$K_{232\ nm}$	$K_{270\ nm}$	$\Delta K_{270\ nm}$
Extra Virgin Olive Oil	≤ 2.5	≤ 0.22	≤ 0.01
Refined Olive Oil + Virgin Olive Oil	N/A	≤ 1.15	≤ 0.15

Table 2. Passing criteria for select olive oil types.

Acceptable limits for $K_{232\ nm}$, $K_{270\ nm}$ and $\Delta K_{270\ nm}$, for both extra virgin olive oil and a mixture of refined and virgin olive oils, as outlined by IOC,[4] are included in Table 2. These limitations are much stricter for extra virgin olive oil than for the olive oil mixture. The later product is expected to be lower in quality and therefore have a greater concentration of oxidation products present versus the former. Table 3 includes the calculated $K_{232\ nm}$, $K_{270\ nm}$ and $\Delta K_{270\ nm}$ values for the five different oil samples. These calculations were carried out using the advanced calculations functionality as shown in Figure 2. Additionally, pass/fail tests are also included in Figure 2 to serve as a quick check whether the calculated values are within limits.

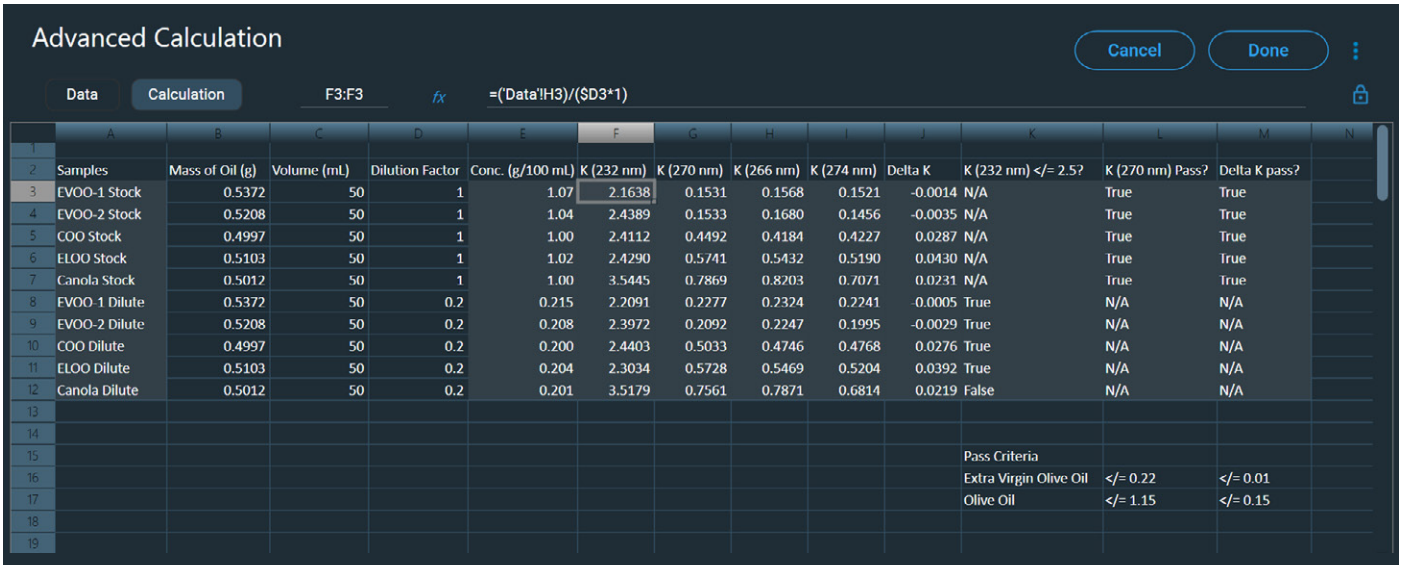
Sample Type	*$K_{232\ nm}$	$K_{270\ nm}$	$\Delta K_{270\ nm}$
EVOO-1	2.21	0.15	0.001
EVOO-2	2.40	0.15	0.004
COO	2.44	0.45	0.029
ELOO	2.30	0.57	0.043
Canola Oil	3.52	0.79	0.023

* Calculations carried out using absorbance at 232 nm of diluted samples as per the experimental section.

Table 3. Calculated K for measured oil samples.

From the calculated specific extinction coefficients (Table 3), both extra virgin olive oil samples are within the bounds outlined in Table 2, implying the samples are not oxidized beyond the permitted limit. The COO and ELOO samples, which are defined as mixtures of refined and virgin olive oil, pass the criteria for $K_{232\ nm}$ for extra virgin olive oil; however, the specific extinction coefficient at 270 nm is unable to meet the criteria for extra virgin olive oil. If the ELOO and COO samples were initially prepared the same as the EVOO-1 and EVOO-2 samples, they would be considered too oxidized by these standards. However, as the calculated $K_{270\ nm}$ and $\Delta K_{270\ nm}$ values are within the limits expected for mixtures of refined and virgin olive oils, these samples do not contain more than the accepted concentration of oxidized byproducts.

The canola oil sample, which contains no olive oil, is unable to meet the requirements for extra virgin olive oil as expected. However, it is able to meet the requirements for $K_{270\ nm}$ and $\Delta K_{270\ nm}$ for the refined/virgin olive oil mixture. As there should be no olive oil present in these samples, these results further highlight that this specific analysis is unable to be used as an identification method for the type of oil. Consequently, additional testing is needed in order to confirm the type of olive oil or determine the presence of oil from a different oil, like canola. For example, chromatographic methods are outlined to determine the presence of extraneous oils as per IOC.[7]



	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1														
2	Samples	Mass of Oil (g)	Volume (mL)	Dilution Factor	Conc. (g/100 mL)	K (232 nm)	K (270 nm)	K (266 nm)	K (274 nm)	Delta K	K (232 nm) </= 2.5?	K (270 nm) Pass?	Delta K pass?	
3	EVOO-1 Stock	0.5372	50	1	1.07	2.1638	0.1531	0.1568	0.1521	-0.0014	N/A	True	True	
4	EVOO-2 Stock	0.5208	50	1	1.04	2.4389	0.1533	0.1680	0.1456	-0.0035	N/A	True	True	
5	COO Stock	0.4997	50	1	1.00	2.4112	0.4492	0.4184	0.4227	0.0287	N/A	True	True	
6	ELOO Stock	0.5103	50	1	1.02	2.4290	0.5741	0.5432	0.5190	0.0430	N/A	True	True	
7	Canola Stock	0.5012	50	1	1.00	3.5445	0.7869	0.8203	0.7071	0.0231	N/A	True	True	
8	EVOO-1 Dilute	0.5372	50	0.2	0.215	2.2091	0.2277	0.2324	0.2241	-0.0005	True	N/A	N/A	
9	EVOO-2 Dilute	0.5208	50	0.2	0.208	2.3972	0.2092	0.2247	0.1995	-0.0029	True	N/A	N/A	
10	COO Dilute	0.4997	50	0.2	0.200	2.4403	0.5033	0.4746	0.4768	0.0276	True	N/A	N/A	
11	ELOO Dilute	0.5103	50	0.2	0.204	2.3034	0.5728	0.5469	0.5204	0.0392	True	N/A	N/A	
12	Canola Dilute	0.5012	50	0.2	0.201	3.5179	0.7561	0.7871	0.6814	0.0219	False	N/A	N/A	
13														
14														
15											Pass Criteria			
16											Extra Virgin Olive Oil	</= 0.22	</= 0.01	
17											Olive Oil	</= 1.15	</= 0.15	
18														
19														

Figure 2. Advanced calculations function in the Insight Pro software for the analysis of various oil samples.

Conclusions

The results outlined herein emphasize the ease at which IOC procedures, which use UV-Visible techniques, can be used to assess olive oil purity. While additional tests are needed to determine overall olive oil quality and authenticity, the ability to detect the presence of oxidation products through non-destructive means outlines the importance of this analysis method for olive oil QA/QC. The ability to carry out this method, including calculations and pass/fail determination, using the Evolution One Plus spectrophotometer and associated Insight Pro software highlights its utility and convenience for quality checks.

References

1. Torrecilla, J. S.; Rojo, E.; Domínguez, J.C.; Rodríguez, F.; A novel method to quantify the adulteration of extra virgin olive oil with low-grade oils by UV-vis, *J. Agric. Food Chem.*, **2010**, 58, 1679 – 1684.
2. Tarapoulouzi, M.; Agriopoulou S.; Koidis, A.; Proestos, C.; Enshasy, H. A. E.; Varzakas, T., Recent advances in analytical methods for the detection of olive oil oxidation status during storage along with chemometrics, authenticity and fraud studies, *Biomol.*, **2022**, 12, 1180.
3. Gargouri, B.; Zribi, A.; Bouaziz, M., Effect of containers on the quality of Chemlali olive oil during storage, *J. Food Sci. Technol.*, **2015**, 52, 1948 – 1959.
4. International Olive Council, Trade Standard Applying to Olive Oils and Olive Pomace Oils, **2019**, COI/T.15/NC No 3/Rev. 13.
5. Jolayemi, O. S.; Tokatli, F.; Ozen, B., UV-Vis spectroscopy for the estimation of variety and chemical parameters of olive oils, *J. Food Meas. Charact.*, **2021**, 15, 4138 – 4149.
6. International Olive Council, Method of Analysis Spectrophotometric Investigation in the Ultraviolet, **2019**, COI/T.20/Doc. No 19/Rev. 5.
7. International Olive Council, Global Method for the Detection of Extraneous Oils in Olive Oil, **2013**, COI/T.20/Doc. No 25/Rev. 1.

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