APPLICATION NOTE

DETERMINING THE PURITY OF OLIVE OIL SAMPLES USING UV-VISIBLE SPECTROSCOPY

INTRODUCTION

The olive trees are a traditional crop of the Mediterranean landscape with olive oil being the primary source of fat and one of the major components of the Mediterranean diet [1]. The major components of olive oil are the fatty acids, of which oleic acid (mono-unsaturated fatty acid) represents 55 to 83%, linoleic acid (poly-unsaturated fatty acid) represents 4 to 21% and palmitic acid (saturated fatty acid) represents 7.5 to 20%, see **Figure 2**.

Owing to the high amount of mono-unsaturated fatty acids, olive oil has antioxidant and anti-inflammatory properties and is essential to reduce bad cholesterol levels, therefore decreasing the risk of cardiovascular diseases [2].

The International Olive Council regulates the different qualities of olive oil, a parameter that can be determined by the amount of acidity. The acidity measures the rate in weight of the free fatty acids

Keywords:

UV-Visible Spectroscopy

DWHP Light Source

FLEX Spectrometer

Olive Oil Samples

Monounsaturated Fatty Acids

Figure 1 – Absorbance Configuration

The highest quality olive oil is classified as extra-virgin and is a chemically unprocessed olive oil that never exceeds an acidity level of 0.8 %. Virgin olive oil, on the other hand, has an acidity level that should never exceed 2 degrees. Olive oil with an acidity level higher than 2% is termed lampante and is not suitable for consumption [3].

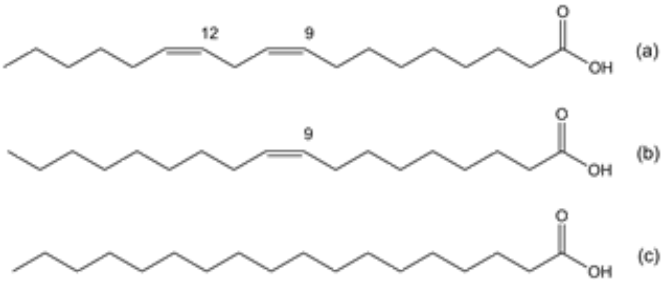

Figure 2 – SLinoleic (a), Oleic (b) and Palmitic (c) fatty acids molecular structure.

Oxidation is a deteriorative process that involves primarily unsaturated lipids and plays an important role in lowering the oil quality. The primary products in unsaturated lipid oxidation show diene or triene conjugated double bonds that result from the changes in the linoleic acyl groups.

These changes in the acyl groups modify the properties of the fatty acids, therefore affecting the olive oil quality. The oxidized fatty acids products show a strong absorption at around 270 nm. For the assessment of the olive oil quality, EEC Regulation 2568/91 (1991) outlines a method using UV-Visible spectroscopy [4].

In this application note, we combine the DWHP light source with a FLEX spectrometer in an UV-Visible configuration to determine the quality of three samples of labeled extra virgin olive oil.

MATERIALS & METHODS

Reagents

- Three Samples of labeled extra virgin olive oil;
- 2,2,4-Trimethylpentane (C8H18, iso-octane, spectroscopic grade > 99.5%, Alfa Aeser);

Instruments and Accessories:

Absorbance Configuration

(see **Figure 1**)

- DWHP light source;
- 400 µm diameter illumination optical fiber;
- Standard cuvette holder set into an absorbance configuration;
- 200 µm diameter collecting optical fiber;
- FLEX STD UV/Vis spectrometer (Slit: 10 µm)
- 10x10 mm absorption cuvettes in UV quartz;

EXPERIMENTAL PROCEDURE

1. Weight 0.25g of an homogeneous solution of an olive oil sample into a 25 mL volumetric flask and make up to the mark with iso-octane.

2. Take the resulting solution that must also be completely homogeneous and measure the absorption spectra between 220 and 300 nm, using iso-octane as a reference.

3. The LightScan software was used with the instruments settings specified in Table 1.

Table 1 – Instrument settings used for experimental absorption measurements.

Parameter	Used Settings
Integration time (ms)	4
Average	300
Smoothing	2

4. The quality of the olive oil sample can be determined from the extinction coefficient K_λ at four different wave¬lengths, (232, 266, 270 and 274 nm), according to the following equations

$$K_\lambda = A_\lambda / (c \times l)$$

and

$$\Delta K = K_{270} - (K_{266} + K_{274}) / 2$$

where A_λ is the absorbance at the wavelength λ, c is the concentration of the sample solution (in g/100 mL) and l is the path length of the absorption cuvette (in cm) [4]. The experimental values obtained from both equations are compared with the maximum permitted K_λ and ΔK values according to the Regulation (EU) 2016/2095 (this regulation amends the Regulation (EEC) No 2568/91 on the characteristics of olive oil), see **Table 2**.

Table 2 – Maximum permitted values for UV-Visible spectroscopy analysis according to Regulation EU 2016/2095 [3].

Olive Oil Quality	Acidity (%)	K_{232}	K_{270}	ΔK
Extra Virgin	≤ 0.8	≤ 2.5	≤ 0.22	≤ 0.01
Virgin	≤ 2.0	≤ 2.6	≤ 0.25	≤ 0.01

RESULTS

The absorption spectra of the three extra virgin olive oil samples in iso-octane are given in Figure 3.

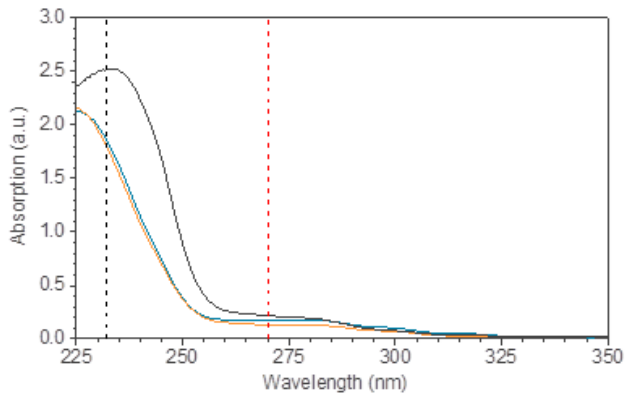

Figure 3 – Absorption spectra of the extra virgin olive oil samples in iso-octane. The black and red vertical dashed lines indicates the 232 and 270 nm, respectively.

In order for an olive oil to be given the extra vir¬gin category, K_232 and K_270 values must be lower than 2.5 and 0.22, respectively, see Table 2.

Additionally, is also essential to get a ΔK lower than 0.01. The evaluation of the absorption spectra of the three olive oil samples according to equations 1 and 2 yields the values given in Table 3.

Table 3 – UV-Visible spectroscopy parameters obtained for three samples of olive oil in iso-octane.

Olive Oil	K_{232}	K_{270}	ΔK
Sample 1	1.85 ± 0.02	0.180 ± 0.01	0.003
Sample 2	1.79 ± 0.01	0.129 ± 0.01	0.001
Sample 3	2.50 ± 0.01	0.220 ± 0.01	0.001

The extinction coefficient values presented in **Table 3** show that all olive oil samples fulfill the criteria given in **Table 2** and, therefore, can be classified as extra virgin. Despite being classified as extra virgin, sample 3 extinction coefficient values are in the border between the extra virgin and virgin category. This means that sample 3 has a higher amount of free fatty acids than samples 1 and 2.

CONCLUSIONS

Sarspec's DWHP light source and FLEX spectrometer were combined in an UV-Visible configuration to ascertain the quality of three extra virgin olive oil samples. The results obtained show that two of three olive oil samples have low absorption values and hence fulfill the criteria from Regulation (EU) 2016/2095 to be classified as extra virgin. The third sample is also classified as an extra virgin. However, the absorption values obtained are very close to the border of the extra virgin with the virgin olive oil category.

This application note demonstrates that the quality and state of preservation of any olive oil can be quick, easy, and accurately measured by using Sarspec's UV-Visible configuration if the experimental procedure and criteria set by the International Olive Council are followed. This application is also important to producers to detect any changes brought about in the olive oil by the technological processes.

REFERENCES

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3. Commission Delegated Regulation (EU) 2016/2095 – Annex I (http://eur-lex.europa.eu/)

4. Commission Regulation (EEC) No 2568/91 – Annex IX (http://eur-lex.europa.eu/)