Determination of Adulterants in Olive Oil

Analysis of Waxes and Fatty Acid Methyl and Ethyl Esters with Capillary GC and Agilent OpenLAB Software

Application Note

Food Testing and Agriculture

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Abstract

The potential benefit of extra virgin olive oil (EVOO) to human health, combined with its high cost, has caused recent increases in the adulteration of this commodity. Therefore, analysis to check the quality of EVOO has become essential to identify the addition of lower-cost seed oils or olive-pomace oil (OPO). This application note describes a method to detect this type of adulteration of EVOO. It is based on measurement of wax ester distribution, and shows how Agilent OpenLAB software can handle many different chromatographic peaks, and automatically calculate key group coefficients that can be used as markers of authenticity and purity of EVOO.

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Introduction

To classify extra virgin olive oil (EVOO) sold in the Brazilian market, the Ministry of Agriculture, Livestock, and Supply (Ministério da Agricultura, Pecuária e Abastecimento, MAPA) published Normative instruction Nº 1 that defines official standards to classify olive oil and olive-pomace oil in relation to their identity and quality, sampling, presentation, and labeling.

This legislation classifies olive oils and olive-pomace oil based on parameters such as the raw material, production process, acidity percent, and technology applied for extraction. There are five classes:

- Virgin olive oil
    a. Extra virgin
    b. Virgin
    c. Lampante
- Olive oil
- Refined olive oil
- Olive-pomace oil
- Refined olive-pomace oil

The legislation states that classification can be verified by measuring the content of waxes and fatty acid ethyl and methyl ester (FAEE and FAME) analyzed by the method described by the International Olive Council (IOC) standards, Doc. No.28. The method is recommended as "a tool for distinguishing between olive oil and olive-pomace oil, and as a quality parameter for extra virgin olive oils enabling the detection of fraudulent mixtures of extra virgin olive oils with lower quality oils whether they are virgin, ordinary, lampante or some deodorized oils" [3].

Wax esters are found in the skin of olives. Cold-pressed EVOO contains only small quantities of these compounds (usually less than 100 mg/kg) [1]. Low-quality olive oil obtained from olive pomace by extraction with heat or solvent, or both, contains much higher quantities of wax esters.

According to the Brazilian legislation, if the amount of wax esters is below or equal to 250 mg/kg, the oil can be classified as EVOO or just virgin olive oil (VOO). To be classified as EVOO, besides the wax parameter limit of 250 mg/kg, the FAME plus FAEE content should be below or equal to 75 mg/kg, or between 75 and 150 mg/kg if the FAME/FAEE ratio is below 1.5. This second FAME and FAEE requirement also tests for freshness because these compounds are formed from degrading olives when they ferment to produce methanol and ethanol, which transesterify with the fatty acids from the triglycerides in the olives [4].

Products with higher wax content can be classified as lampante if the wax content is still below or equal to 300 mg/kg, and as olive oil or refined olive oil if still below or equal to 350 mg/kg. Olive-pomace oil has levels higher than 350 mg/kg, as does refined olive-pomace oil.

Hence, high contents of wax and esters in VOO can indicate its adulteration with lower-quality oils.

Experimental

Apparatus and reagents

- Glass column with 15 g silica
- Individual standard solutions of C40 (behenyl oleate), C42 (behenyl arachidate), and C44 (behenyl behenate) prepared in heptane, from Larodan Fine Chemicals
- Standard solution of lauryl arachidate at 0.05% (m/V) in heptane (internal standard for waxes) from Sigma-Aldrich, Corp.
- Standard solution of heptadecanoate at 0.02% (m/V) in heptane (internal standard for methyl and ethyl esters) from Sigma-Aldrich, Corp.
- HPLC grade ethyl ether, hexane, and heptane from Sigma-Aldrich, Corp.
- Sudan I (1-phenylazo-2-naphthol) from Sigma-Aldrich, Corp., 1% in water, used for a visual check to see if the waxes eluted properly
- Rotary evaporator

Instrumentation

Conditions

GC:	Agilent 7890A series (G3440A)
Column:	Agilent J&W HP-5, 10 m × 0.32 mm, 0.25 µm (p/n 19091J-413) cut with 5 m retention gap
Inlet:	On-column
Injection volume:	1 µL
Carrier gas:	Helium
Oven temperature program:	80 °C (1 min), 2 °C/min to 140 °C (0 min), 7 °C/min to 335 °C (30 min)
Inlet temperature:	Oven track function
Detector:	FID at 350 °C
Software:	Agilent OpenLAB ChemStation Data Analysis

Sample preparation

The sample was prepared as shown in Figure 1 using the IOC method [3].

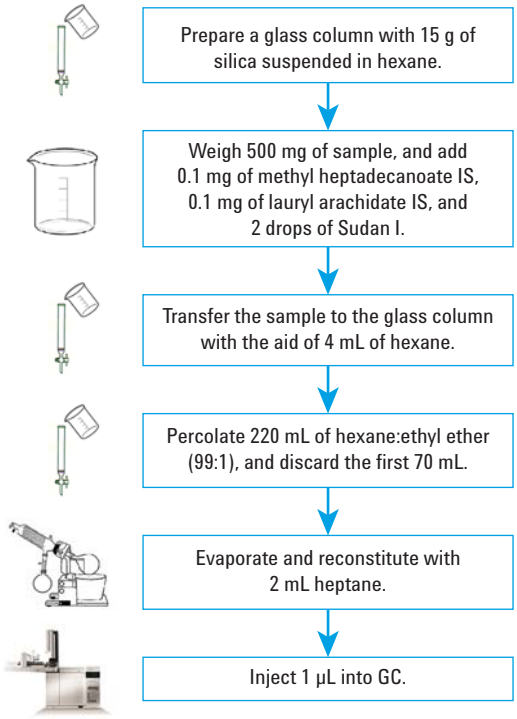

Figure 1. Workflow to prepare olive oil samples for GC analysis according to the IOC.

Results and Discussion

Figure 2 shows a chromatogram of a sample labeled as EVOO purchased at a local supermarket. There are two methyl esters and two ethyl esters that are critical to the FAME and FAEE calculations. These were well resolved and measurable, along with their shared internal standard of methyl heptadecanoate. The wax esters C40, C42, C44, and C46 were similarly well resolved with their internal standard of lauryl arachidate. The identification of the waxes was also controlled with the aid of an individual standard solution of C40, C42, and C44, as described by IOC.

The Intelligent Reporting function in Agilent OpenLAB ChemSation Data Analysis software automatically calculates the total amount of wax (C40, 42, 44, and 46), FAEE (C16E and C18E) and FAME (C16M and C18M) expressed in mg/kg, considering the weight of the sample as 500 mg and weights of internal standards (0.05 mg methyl heptadecanoate for each ester calculation and 0.1 mg lauryl arachidate for wax calculation).

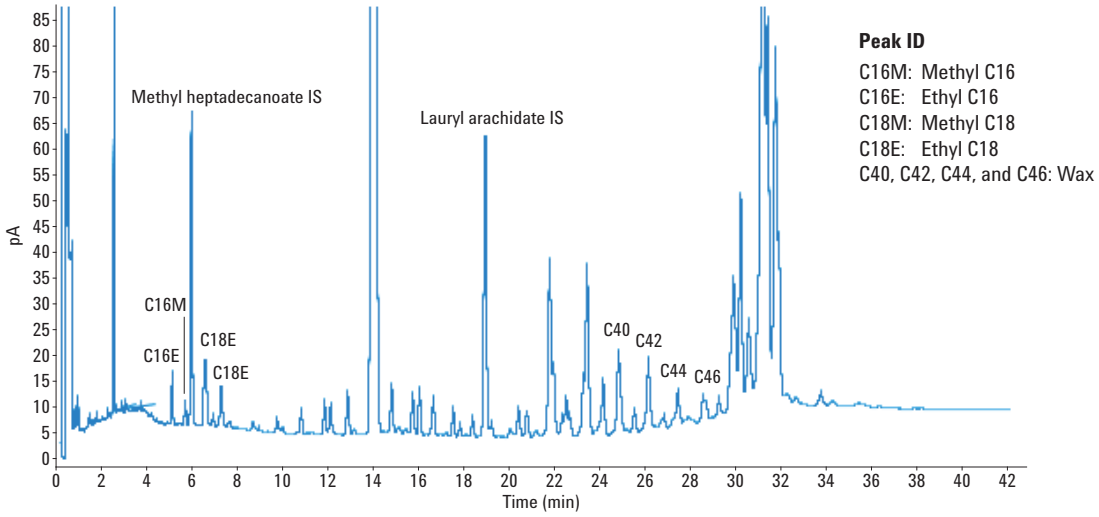

Figure 2. Chromatogram of an olive oil labeled as extra virgin, showing good resolution of critical ethyl and methyl esters.

The content of each alkyl ester, in mg/kg of fat, is calculated using Equation 1 [3].

Equation 1.

$$\text{Ester, mg/kg} = \frac{(Ax)(Ms)1000}{(As)(m)}$$

Ax = Area corresponding to the peak C16 and C18 ester
As = Area corresponding to the peak of the methyl heptadecanoate internal standard
Ms = Mass of the methyl heptadecanoate internal standard added, in mg
m = Mass of the sample taken for determination, in g

The content of wax, in mg/kg of fat, is calculated using Equation 2 [3].

Equation 2.

$$\text{Waxes, mg/kg} = \frac{(\Sigma Ax)(Ms)1000}{(As)(m)}$$

Ax = Area corresponding to C40, C42, C44, and C46
As = Area corresponding to the peak of the lauryl arachidate internal standard
Ms = Mass of the lauryl arachidate internal standard added, in mg
m = Mass of the sample taken for determination, in g

OpenLAB is able to present the results of these measurements and calculations in a summary report, which makes it easy to check peak identification and to check classification of samples (for example, EVOO or not).

In the example given in Figure 3, the total content of FAME and FAEE was between 75 and 150 mg/kg (89.24 mg/kg) and the ΣFAEE/ΣFAME ratio was below 1.5. The wax content was below the limit established by legislation, <250 mg/kg. According to the results, this sample can be considered as EVOO.

Single Injection Report

IS ester	RT [min]	Area
IS ester	5.927	3313.8942
	Sum	3313.8942
Methyl Group	**RT [min]**	**Area**
C16M	5.072	508.479
C18M	6.533	832.8063
	Sum	1341.2853
Ethyl Group	**RT [min]**	**Area**
C16E	5.634	641.2831
C18E	7.197	974.6989
	Sum	1615.9820

∑ FAME = 0.02024 mg 40.47 mg/kg

∑ FAEE = 0.02438 mg 48.76 mg/kg

∑ FAEE/∑ FAME = 1.205

∑ FAEE + ∑ FAME = 89.24 mg/kg

IS wax	RT [min]	Area
IS wax	18.825	5347.9482
	Sum	5347.9482
Wax	**RT [min]**	**Area**
C40	24.6	750.4276
C42	25.913	677.0756
C44	27.209	332.7996
C46	28.311	478.6969
	Sum	2238.9996

∑ Wax = 0.04187 mg **83.73** mg/kg

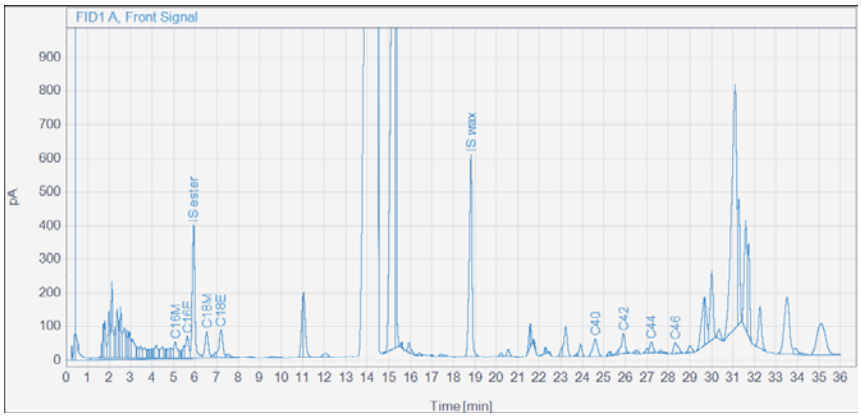

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Figure 3. Example of a report created by Agilent OpenLAB Data Analysis Intelligent Reporter, showing the fatty acid methyl and ester content and ratios in a sample of olive oil.

Conclusions

Classification of olive oil through assessment of key waxes and alkyl esters can be performed with a simple Agilent 7890 GC analysis that follows a reference method from the IOC [3], and which is indicated in government legislation. However, some custom calculations are required. Exporting values to another data format for these calculations can be time consuming, inconvenient, and carries a risk of errors. For a routine laboratory, it is important to mitigate potential errors; therefore, the use of automated calculations and reporting within OpenLAB software is not only practical but essential to ensure submission of reliable results.

References

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