APPLICATION NOTE

Gas Chromatography

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Determination of the Acidic Composition of Triglycerides and *Trans* Fatty Acids

Introduction

The saponifiable fraction of olive oil is composed primarily of triglycerides, consisting of one molecule of glycerol on which are esterified up to three fatty acid chains (all with an even number of carbon atoms) saturated and unsaturated. Since the biosynthesis of fatty acids within the plant is variable depending on the species considered, the presence of fatty acids normally absent in the olive oil is indicative of adulteration of the product with oils obtained from different seeds. In particular, the relative percentages of myristic, linolenic, arachidic, eicosenoic, behenic and lignoceric acids are measured, as specified in EC Regulation 2568/91. The percentage composition of the other fatty acids commonly present in olive oil (oleic, linoleic, palmitic, etc.) is also used for determining the ECN42, in the calculation of theoretical composition of triglycerides. The chromatographic separation on an appropriate capillary column allow to separate and quantify not only the normal (cis) fatty acid, but also their *trans*-isomers. These compounds are important markers of heat treatments (deodorization) or refining (e.g. activated carbons). The official methods of this analysis are described in Annex X of EU Regulation 2568/91.

Principle of the Method

Before proceeding to the determination of fatty acids by GC-FID, a glycerides hydrolysis should be performed to cleave the free fatty acids linked to glycerol. In this way, the fatty acids can be transformed (by a process of trans-esterification with methanol in basic conditions) in their respective methyl esters, which have a higher volatility and a lower polarity.

Methylated fatty acids can be injected into the column, separated and detected with FID. In this way it is possible to obtain a profile of the acidic composition of the oil in terms of fatty acid-derived methyl esters (FAMEs). The identification and quantification of individual fatty acids are carried out by comparing areas and retention times with those of the internal standard.

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Sample Preparation

A possible sample preparation is described in the A Method (cold trans esterification) reported in the Annex X B of EC Regulation 2568/91 and can be summarized in the following steps:

1. Weigh 0.1 g of sample
2. Add 2 mL of heptane
3. Add 0.2 mL of 2 N methanolic KOH
4. Shake and recover the supernatant after stratification of the phases.

Table 1. Instrumentation.

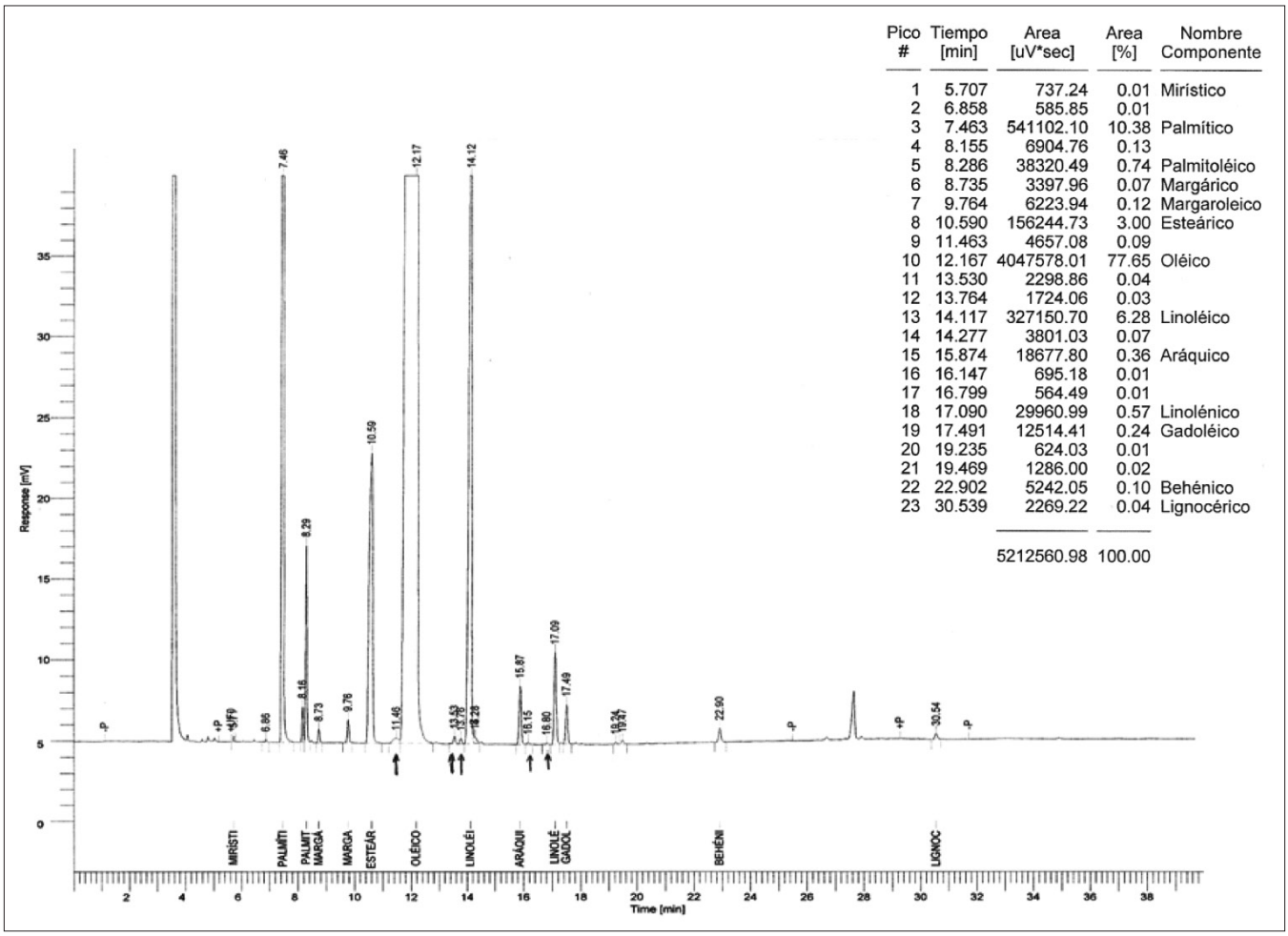
Gas Chromatograph:	Clarus 580 GC Electronic Flow Control
Injector:	Split/Splitless Injector
Detector:	FID Flame Ionization Detector
Software:	TotalChrom® Computing and Instrumentation Management Software
Columns:	Elite Column, 60 m; 0.25 µm, 0.25 mm Part No. N9316508

Table 2. Analytical Method.

Injection Volume:	1.0 µL
Injector Temperature:	200 °C
Column Temperature:	300 °C
Heating Ramp:	170 °C (held 13 min) 1.5 °C/min up to 190 °C 190 °C (held 10 min)

From the chromatogram analysis, it is possible to calculate the percentage composition of some Fatty Acids as required by the Regulation (Myristic, Linolenic, Arachidic, Eicosenoic, Behenic, and Lignoceric Acids). It is also possible to determine and express their concentration as a percentage of total fatty acids to calculate:

- The sum of TRANS isomers of Oleic Acid (C18:1);
- The sum of TRANS isomers of Linoleic and Linolenic Acids (C18:2 and C18:3).



Pico #	Tiempo [min]	Area [uV*sec]	Area [%]	Nombre Componente
1	5.707	737.24	0.01	Mirístico
2	6.858	585.85	0.01	
3	7.463	541102.10	10.38	Palmítico
4	8.155	6904.76	0.13	
5	8.286	38320.49	0.74	Palmitoléico
6	8.735	3397.96	0.07	Margárico
7	9.764	6223.94	0.12	Margaroleico
8	10.590	156244.73	3.00	Esteárico
9	11.463	4657.08	0.09	
10	12.167	4047578.01	77.65	Oléico
11	13.530	2298.86	0.04	
12	13.764	1724.06	0.03	
13	14.117	327150.70	6.28	Linoléico
14	14.277	3801.03	0.07	
15	15.874	18677.80	0.36	Aráquico
16	16.147	695.18	0.01	
17	16.799	564.49	0.01	
18	17.090	29960.99	0.57	Linolénico
19	17.491	12514.41	0.24	Gadoléico
20	19.235	624.03	0.01	
21	19.469	1286.00	0.02	
22	22.902	5242.05	0.10	Behénico
23	30.539	2269.22	0.04	Lignocérico
		5212560.98	100.00	

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