

APPENDIX 1. Estimation of FA profile

The fatty acid compositional profile was estimated with a spreadsheet which permits to calculate the mass fraction of each FA from the mass fraction of each TAG previously measured. This was calculated by applying a mass balance and summarizing all the FA mass fractions found in each TAG. A total of the contributions of the different TAGs results in the fraction of the specific fatty acid. For example, if the mole fraction of oleic acid (O) in 1,2-olein-3-palmitin (OOP) is 2/3, and its abundance in the sample of "Picual" variety is 25.99% (w/w) (Table A1), therefore the contribution of this TAG to the O mass fraction in this sample will be $2 \times 25.99 \times (M_O / M_{OOP})$, which is 17.18% (w/w); M_O and M_{OOP} are the molecular mass of O and OOP respectively. A similar estimation methodology has been also applied by Chapagain and Wiesman¹, however an improvement has been added, by introducing the molecular weights of each FA or TAG in the calculations.

This semi-quantitative methodology would be just valid for virgin olive oil (where the sum of the total contents of free fatty acids plus waxes have to be smaller than 1%) because other FA sources (free FA, waxes) are not being considered and the results are only obtained from the bonded FAs as TAGs.

The next aspect regards to the determination of the FAs in the olive oils from the TAG compositional profile. The major FAs (on a percent basis larger than 1%) from the ten monovarietal extra virgin olive oils are shown in Table A1.

TABLE A1

All oil samples demonstrated typical fatty acids profiles. As expected, oleic acid (18:1) was the most dominant fatty acid by far, followed by palmitic (16:0) and linoleic acid (C18:2). However, significant variations were found among the different varieties of extra virgin olive oil, in all FAs. The highest level of oleic acid was found in the sample matching to "Manzanilla" (77.14%) and the lowest in the "Arbequina" (67.82%) one. Moreover, the highest level of palmitic acid was found in the "Empeltre" sample (22.22%) and the lowest in the "Cornicabra" (11.05%) one. Nevertheless, the sample corresponding to "Blanqueta" showed the lowest level in stearic acid (1.33%) and the "Hojiblanca" one the highest level (3.48%). These results shows that the compositional distribution of fatty acids of these oil

¹ P. Bishnu, Z. Wiesman, *J. Agric. Food Chem.* 2009, 57, 1135-1142.

samples are within the normal range expected for olive oil as defined by IOOC standard², and EU regulation³.

Next, in the tables A2 and A3, the obtained results of the two external reference materials are shown. It has been taking into account the TAG PoOO and the mass fraction of POL has been recalculated, since they both might be co-eluted, and consequently the percentage of palmitic, linoleic and oleic acid may be different.

As it can be seen in both tables the value of palmitic and linoleic acid decrease due to the drop in the percentage of POL; on the other hand the mass fraction of oleic acid increase due to the existence of PoOO.

² COI/T.15/NC no.3/Rev.1. Trade standard applying to olive oil and olive-pomace oils. International Olive Oil Council (IOOC), 2003.

³ COMMISSION REGULATION (EC) No 702/2007 amending Commission Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive residue oil and on the relevant methods of analysis, *Off. J. Eur. Union*, 2007, L 161/11-27.

Table A1. Fatty acid profile of extra virgin olive oils from ten Spanish olive oil varieties.

Olive oil variety	FA Composition (%)			
	P (C16:0) ^a	S (C18:0) ^b	O (C18:1) ^c	L (C18:2) ^d
Arbequina	21.4	1.5	67.8	9.3
Cornicabra	11.1	3.2	78.8	7.1
Empeltre	22.2	1.7	68.9	7.2
Picual	15.0	3.2	75.2	6.7
Hojiblanca	14.8	3.5	75.5	6.2
Manzanilla	12.3	2.9	79.1	5.6
Blanqueta	18.5	1.3	71.9	8.3
Verdial	13.2	3.3	77.2	6.4
Picudo	12.7	2.8	78.5	6.1
Lechín	14.1	3.1	75.5	7.3

^a palmitic acid; ^b stearic acid; ^c oleic acid; ^d linoleic acid.

Table A2. Comparison of the major FA obtained for the olive oil external reference material (EVOO A) with the reference value. Calculations with PoOO are shown in the last row.

	% L	% O	% P	% S	% Po
Reference value	5.58	77.72	10.80	3.46	0.88
Initial obtained value	5.9	74.6	12.0	3.1	-
Recalculated with PoOO	5.0	76.3	11.1	3.1	0.9

Table A3. Comparison of the major FA obtained for the olive oil external reference material (EVOO B) with the reference value. Calculations with PoOO are shown in the last row.

	% L	% O	% P	% S	% Po
Reference value	5.80	77.33	11.23	3.12	0.88
Initial obtained value	5.9	74.6	12.9	2.5	-
Recalculated with PoOO	4.9	75.9	12.0	2.6	0.9