

Supplementary Information

Pilot Study on Adulteration in Extra Virgin Olive Oil: A Multiblock Approach to Relate Gas Chromatography, Digital Imaging, and Spectroscopy

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Table S1. Absorbance values read at 456 nm for all samples analyzed

Sample	Absorbance at 456 nm
P	0.9377 ± 0.0283 ^{be}
S1	0.9270 ± 0.0229 ^e
S5	0.8783 ± 0.0316 ^m
S10	0.7937 ± 0.0160 ^k
S20	0.6923 ± 0.0357 ^l
S50	0.4403 ± 0.0015 ^d
S70	0.1787 ± 0.0012 ^j
S90	0.0250 ± 0.0089 ⁿ
SO	ND
1	1.2243 ± 0.0098 ^g
2	1.6450 ± 0.0106 ^f
3	1.2360 ± 0.0060 ^g
4	1.8600 ± 0.0151 ^h
5	1.3460 ± 0.0010 ⁱ
6	1.2010 ± 0.0036 ^g
7	1.6697 ± 0.0012 ^f
8	1.4887 ± 0.0081 ^a
9	1.2267 ± 0.0015 ^g
10	1.5227 ± 0.0085 ^a
11	0.9737 ± 0.0025 ^b
12	1.7830 ± 0.0017 ^c
13	1.5087 ± 0.0084 ^a
14	0.4367 ± 0.0012 ^d
15	0.9387 ± 0.0012 ^{be}
CO	0.1750 ± 0.0078 ^j

ND: not detectable. Results expressed as mean ± SD (standard deviation) of triplicate, absorbance values read at 456 nm. Values with different lowercase letters in the same column are significantly different ($p < 0.05$) by Tukey's test.

Table S2. Fatty acid composition values for all samples analyzed, according to the International Olive Council specification¹

Sample	16:0	16:1n-9	16:1n-7	18:0	18:1n-9	18:1n-7	18:2n-6	18:3n-6	18:3n-3	20:0	22:0
P	14.15 ±	0.0762 ±	1.309 ±	2.200 ±	66.43 ±	3.472 ±	11.04 ±	0.6374 ±	0.3630 ±	0.2361 ±	0.0843 ±
	0.0145 ^{ab}	0.0117 ^{abcde}	0.1103 ^{ek}	0.0041 ^{adef}	0.1519 ^h	0.0221 ^{de}	0.0388 ^{gl}	0.0076 ^{abc}	0.0265 ^{abc}	0.0288 ^{abcdefgk}	0.0593 ^{bd}
S1	14.22 ±	0.0487 ±	1.204 ±	2.166 ±	65.86 ±	3.196 ±	11.81 ±	0.7240 ±	0.3845 ±	0.2623 ±	0.1231 ±
	0.1544 ^{ab}	0.0048 ^{defgi}	0.0453 ^{klm}	0.0720 ^{cdef}	0.5879 ^h	0.0899 ^{cij}	0.6808 ^{hl}	0.0495 ^{bc}	0.0166 ^{abc}	0.0100 ^{aceghik}	0.0162 ^{cd}
S5	14.14 ±	0.0530 ±	1.199 ±	2.254 ±	64.75 ±	3.219 ±	12.79 ±	0.8251 ±	0.3839 ±	0.2510 ±	0.1325 ±
	0.1560 ^{ab}	0.0073 ^{adefg}	0.0253 ^{aklm}	0.1552 ^{aef}	0.1938 ^k	0.1077 ^{cij}	0.1010 ⁱ	0.0187 ^c	0.0025 ^{abc}	0.0075 ^{acdeghk}	0.0079 ^{cd}
S10	13.93 ±	0.0403 ±	1.107 ±	2.375 ±	62.67 ±	3.128 ±	14.87 ±	1.068 ±	0.3915 ±	0.2560 ±	0.1629 ±
	0.1066 ^{ab}	0.0120 ^{fgij}	0.0910 ^{abl}	0.1495 ^{abef}	0.1999 ^b	0.0534 ^{bij}	0.1623 ^b	0.0323 ^f	0.0016 ^{abc}	0.0041 ^{aceghk}	0.0101 ^c
S20	13.66 ±	0.0580 ±	1.052 ±	2.546 ±	59.75 ±	3.023 ±	17.72 ±	1.354 ±	0.4050 ±	0.2491 ±	0.1834 ±
	0.0629 ^{ac}	0.0072 ^{adefg}	0.0368 ^{bc}	0.0465 ^{ab}	0.2121 ^j	0.0398 ^{abj}	0.2256 ^m	0.0122 ^g	0.0095 ^{abc}	0.0058 ^{abcdeghk}	0.0055 ^c
S50	12.66 ±	0.0408 ±	0.6610 ±	3.258 ±	47.57 ±	2.424 ±	29.77 ±	2.581 ±	0.4313 ±	0.2562 ±	0.3433 ±
	0.0644 ^{hkm}	0.0039 ^{efgij}	0.0262 ^o	0.1378 ^h	0.2668 ^l	0.0411 ^{km}	0.1918 ⁿ	0.0346 ^h	0.0222 ^{bc}	0.0079 ^{aceghk}	0.0043 ^e
S70	11.96 ±	0.0304 ±	0.3934 ±	3.845 ±	40.46 ±	2.105 ±	36.86 ±	3.319 ±	0.4422 ±	0.2009 ±	0.3798 ±
	0.0929 ^{lmn}	0.0039 ^{ghij}	0.0226 ⁱ	0.0648 ^g	0.0542 ^m	0.0231 ^{gl}	0.0573 ^o	0.0691 ⁱ	0.0170 ^{bc}	0.0317 ^{bdf}	0.0206 ^e
S90	11.45 ±	0.0099 ±	0.1641 ±	4.389 ±	32.20 ±	1.664 ±	44.78 ±	4.153 ±	0.4521 ±	0.2200 ±	0.5117 ±
	0.1618 ^{fgn}	0.0089 ^{hij}	0.0224 ^j	0.0651 ⁱ	0.2294 ⁿ	0.0631 ^h	0.4099 ^p	0.0428 ^j	0.0167 ^c	0.0137 ^{bcdefg}	0.0032 ^f
SO	10.99 ±	ND	0.0723 ±	4.735 ±	27.72 ±	1.449 ±	49.48 ±	4.784 ±	0.4912 ±	0.2327 ±	0.0501 ±
	0.1330 ^{efgn}		0.0075 ^j	0.0580 ⁱ	0.0268 ^f	0.0562 ^h	0.0353 ^e	0.0396 ^e	0.0105 ^{abc}	0.0173 ^{bcd}	0.0114 ^f

Table S2. Fatty acid composition values for all samples analyzed, according to the International Olive Council specification¹ (cont.)

Sample	16:0	16:1n-9	16:1n-7	18:0	18:1n-9	18:1n-7	18:2n-6	18:3n-6	18:3n-3	20:0	22:0
1	14.46 ±	0.1033 ±	1.290 ±	2.332 ±	66.34 ±	3.209 ±	10.99 ±	0.5996 ±	0.3385 ±	0.2877 ±	0.0525 ±
	0.1711 ^{bj}	0.0100 ^c	0.0318 ^{ekm}	0.1640 ^{abef}	0.1745 ^h	0.0649 ^{cij}	0.0775 ^{gl}	0.0210 ^{ab}	0.1020 ^{abc}	0.0035 ^{hijk}	0.0655 ^{ab}
2	14.08 ±	0.0834 ±	1.158 ±	2.499 ±	69.04 ±	3.150 ±	8.737 ±	0.5887 ±	0.4013 ±	0.2532 ±	0.0144 ±
	0.1495 ^{ab}	0.0030 ^{abcd}	0.0433 ^{ablm}	0.0644 ^{abe}	0.1223 ^a	0.0461 ^{ij}	0.0700 ^a	0.0034 ^{ab}	0.0062 ^{abc}	0.0140 ^{acdeghk}	0.0016 ^a
3	13.12 ±	0.0637 ±	0.9418 ±	2.257 ±	69.62 ±	2.632 ±	10.02 ±	0.6169 ±	0.4046 ±	0.3142 ± 0.0099 ^{ij}	0.0150 ±
	0.1113 ^{ch}	0.0091 ^{abdefg}	0.0088 ^{cfg}	0.0070 ^{aef}	0.1058 ^a	0.0546 ^{fk}	0.0238 ^f	0.0304 ^{ab}	0.0213 ^{abc}		0.0020 ^a
4	15.98 ±	0.0813 ±	1.604 ±	2.099 ±	63.22 ±	3.559 ±	12.27 ±	0.5524 ±	0.3692 ±	0.2571 ±	0.0129 ±
	0.1139 ⁱ	0.0064 ^{abcd}	0.0530 ⁿ	0.1187 ^{cdf}	0.1531 ^b	0.0524 ^e	0.0711 ^{hi}	0.0786 ^{ab}	0.0520 ^{abc}	0.0056 ^{acdeghk}	0.0021 ^a
5	15.02 ±	0.0750 ±	1.479 ±	2.086 ±	66.01 ±	3.548 ±	10.56 ±	0.5687 ±	0.3813 ±	0.2663 ±	0.0162 ±
	0.1791 ^j	0.0120 ^{abcdef}	0.0517 ^{dn}	0.1555 ^{cdf}	0.1102 ^h	0.0733 ^e	0.0652 ^{fg}	0.0084 ^{ab}	0.0304 ^{abc}	0.0024 ^{aeghik}	0.0010 ^a
6	11.16 ±	0.0862 ±	0.7057 ±	3.267 ±	73.06 ±	2.117 ±	8.281 ±	0.6532 ±	0.3877 ±	0.2706 ±	0.0148 ±
	0.0601 ^{efg}	0.0059 ^{abc}	0.0216 ^{ho}	0.0995 ^h	0.1917 ^{ci}	0.0402 ^{gl}	0.0395 ^{aj}	0.0043 ^{abc}	0.0316 ^{abc}	0.0028 ^{aghik}	0.0013 ^a
7	12.26 ±	0.0869 ±	0.7998 ±	2.652 ±	73.27 ±	2.543 ±	7.103 ±	0.5884 ±	0.4220 ±	0.2651 ±	0.0131 ±
	0.0485 ^{klm}	0.0102 ^{abc}	0.0420 ^{fho}	0.0299 ^b	0.0631 ⁱ	0.1379 ^k	0.0448 ^{cd}	0.0107 ^{ab}	0.0104 ^{abc}	0.0144 ^{aeghik}	0.0027 ^a
8	12.79 ±	0.0796 ±	0.9497 ±	2.430 ±	71.81 ±	2.820 ±	7.910 ±	0.5481 ±	0.3727 ±	0.2742 ±	0.0150 ±
	0.1012 ^{hk}	0.0178 ^{abcd}	0.0453 ^{cg}	0.0686 ^{abef}	0.1341 ^d	0.0039 ^{af}	0.0328 ^{adj}	0.0322 ^{ab}	0.0119 ^{abc}	0.0136 ^{ahik}	0.0009 ^a
9	11.81 ±	0.0772 ±	0.7979 ±	3.276 ±	72.89 ±	2.249 ±	7.594 ±	0.6471 ±	0.4145 ±	0.2307 ±	0.0115 ±
	0.0971 ^{fln}	0.0144 ^{abcd}	0.0188 ^{fho}	0.0371 ^h	0.0970 ^{ci}	0.0544 ^{lm}	0.0235 ^{cdj}	0.0210 ^{abc}	0.0064 ^{abc}	0.0126 ^{abcdefg}	0.0018 ^a

Table S2. Fatty acid composition values for all samples analyzed, according to the International Olive Council specification¹ (cont.)

	16:0	16:1n-9	16:1n-7	18:0	18:1n-9	18:1n-7	18:2n-6	18:3n-6	18:3n-3	20:0	22:0
10	13.74 ±	0.0854 ±	1.055 ±	2.540 ±	69.75 ±	2.930 ±	8.682 ±	0.6096 ±	0.3674 ±	0.2263 ±	0.0107 ±
	0.1886 ^{abc}	0.0073 ^{abc}	0.0369 ^{abc}	0.1083 ^{ab}	0.2141 ^a	0.0708 ^{ab}	0.1220 ^a	0.0453 ^{ab}	0.0402 ^{abc}	0.0200 ^{abcdefg}	0.0017 ^a
11	14.20 ±	0.0574 ±	1.366 ±	1.874 ±	62.54 ±	3.389 ±	15.21 ±	0.6825 ±	0.3716 ±	0.2911 ±	0.0138 ±
	0.5567 ^{ab}	0.0091 ^{adefg}	0.0074 ^{de}	0.0224 ^{cd}	0.9171 ^b	0.0979 ^{cde}	0.3177 ^b	0.0206 ^{abc}	0.0213 ^{abc}	0.0096 ^{hij}	0.0024 ^a
12	14.19 ±	0.0974 ±	0.8289 ±	1.820 ±	72.27 ±	2.830 ±	6.788 ±	0.5184 ±	0.3695 ±	0.2794 ±	0.0125 ±
	0.7646 ^{ab}	0.0148 ^{bc}	0.0243 ^{fgh}	0.1582 ^c	0.5029 ^{cd}	0.1196 ^{af}	0.0660 ^c	0.0404 ^a	0.0241 ^{abc}	0.0241 ^{ahik}	0.0024 ^a
13	7.308 ±	0.0774 ±	0.5074 ±	2.426 ±	79.08 ±	1.978 ±	7.295 ±	0.5823 ±	0.3961 ±	0.3391 ± 0.0093 ^j	0.0127 ±
	0.0854 ^d	0.0023 ^{abcd}	0.0126 ⁱ	0.0856 ^{abef}	0.0571 ^e	0.0651 ^g	0.1048 ^{cd}	0.0693 ^{ab}	0.0641 ^{abc}		0.0010 ^a
14	11.14 ±	0.0144 ±	0.1173 ±	3.932 ±	28.36 ±	1.533 ±	49.21 ±	5.155 ±	0.3136 ±	0.1973 ±	0.0282 ±
	0.0951 ^{efg}	0.0006 ^{hij}	0.0077 ⁱ	0.1072 ^g	0.1549 ^f	0.0184 ^h	0.0726 ^e	0.1204 ^d	0.0721 ^a	0.0175 ^{bf}	0.0129 ^{ab}
15	10.48 ±	0.0632 ±	1.343 ±	2.404 ±	70.76 ±	3.289 ±	10.56 ±	0.5110 ±	0.3671 ±	0.2138 ±	0.0138 ±
	0.2806 ^e	0.0333 ^{abdefg}	0.0862 ^{dek}	0.2799 ^{abef}	0.3034 ^g	0.0720 ^{cdi}	0.0155 ^{fg}	0.0858 ^a	0.0162 ^{abc}	0.0463 ^{bcdef}	0.0026 ^a
CO	10.78 ±	ND	0.0732 ±	3.950 ±	27.66 ±	1.594 ±	50.81 ±	4.566 ±	0.3311 ±	0.1936 ± 0.0185 ^f	0.0507 ±
	0.1271 ^{eg}		0.0139 ^j	0.0909 ^g	0.0595 ^f	0.0654 ^h	0.2437 ^k	0.0552 ^e	0.0526 ^{ab}		0.0078 ^{ab}

ND: not detected. Results expressed as mean ± SD (standard deviation) of triplicate fatty acid compositions. Values with different lowercase letters in the same column are significantly different ($p < 0.05$) by Tukey's test.

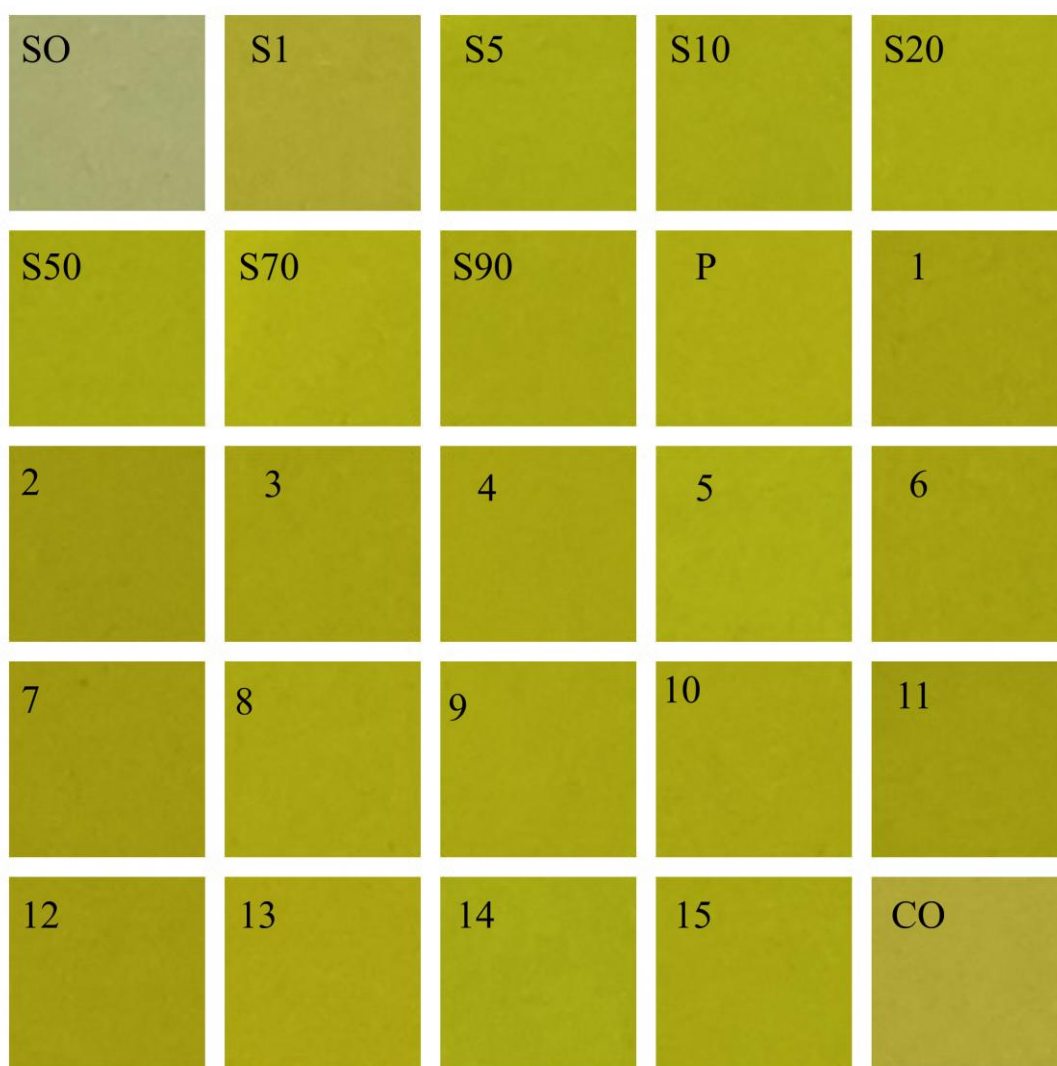


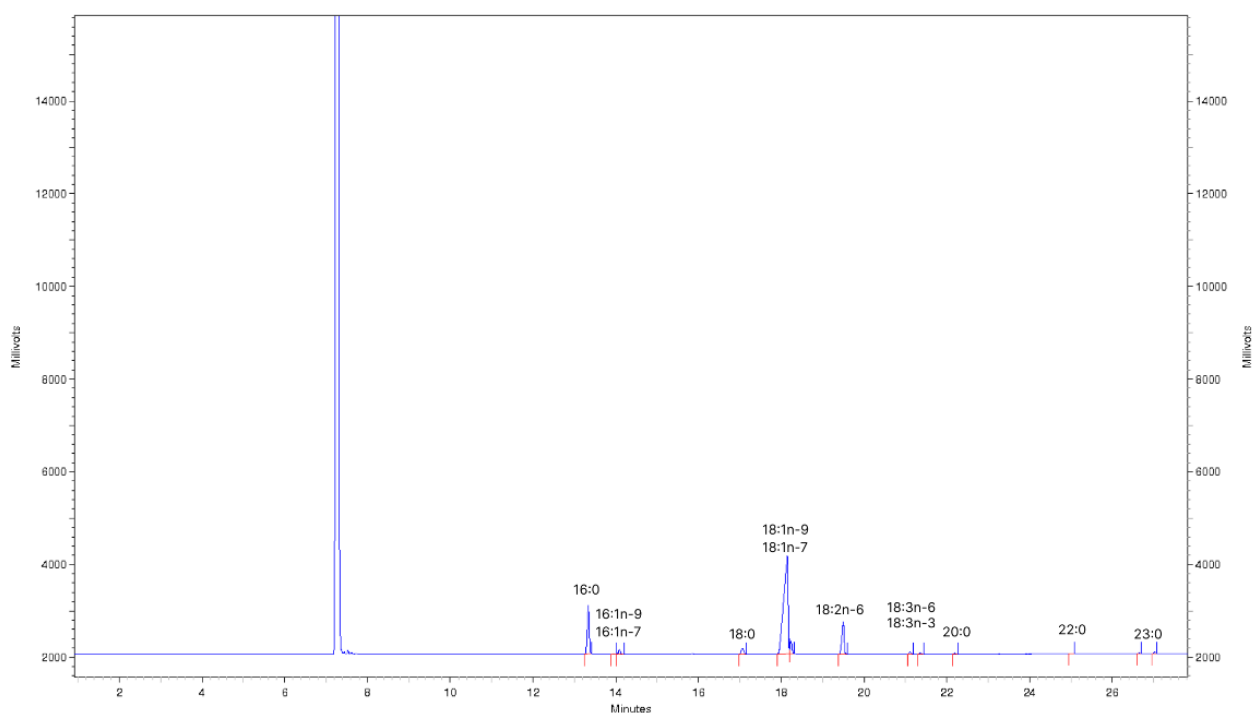
Figure S1. Representation of the images after cropping at a size of 50×50 pixels of the samples. SO: refined soybean oil; S1, S5, S10, S20, S50, S70, S90: adulterations at different levels; P: standard; 1-15: extra virgin olive oil (EVOO); CO: compound oil.

Table S3. Values of color intensity in the R, G, and B channels

Sample	Color channel		
	R	G	B
P	0.674 ± 0.005 ^{aek}	0.670 ± 0.005 ^{jl}	0.074 ± 0.005 ^a
S1	0.659 ± 0.006 ^{abcdei}	0.653 ± 0.007 ^{bghij}	0.071 ± 0.006 ^a
S5	0.649 ± 0.005 ^{bcfgij}	0.649 ± 0.005 ^{bcghi}	0.073 ± 0.005 ^a
S10	0.655 ± 0.007 ^{bcdij}	0.651 ± 0.006 ^{bcghi}	0.072 ± 0.006 ^a
S20	0.666 ± 0.006 ^{abde}	0.667 ± 0.006 ^{ijl}	0.078 ± 0.006 ^a
S50	0.639 ± 0.005 ^{fgj}	0.647 ± 0.005 ^{bcdg}	0.078 ± 0.005 ^a
S70	0.654 ± 0.006 ^{bcfij}	0.666 ± 0.005 ^{hijl}	0.082 ± 0.005 ^a
S90	0.677 ± 0.007 ^{ak}	0.650 ± 0.007 ^{bcghi}	0.203 ± 0.007 ^b
SO	0.667 ± 0.006 ^{adek}	0.671 ± 0.006 ^{jl}	0.435 ± 0.006 ^c
1	0.644 ± 0.006 ^{cfgij}	0.623 ± 0.007 ^{ae}	0.067 ± 0.006 ^a
2	0.619 ± 0.005 ^h	0.592 ± 0.005 ^k	0.068 ± 0.005 ^a
3	0.648 ± 0.005 ^{bcfgij}	0.629 ± 0.005 ^{ade}	0.068 ± 0.005 ^a
4	0.661 ± 0.005 ^{abcde}	0.641 ± 0.005 ^{abcd}	0.070 ± 0.005 ^a
5	0.675 ± 0.005 ^{ak}	0.679 ± 0.005 ^l	0.079 ± 0.005 ^a
6	0.653 ± 0.006 ^{bcfij}	0.633 ± 0.006 ^{acd}	0.070 ± 0.006 ^a
7	0.631 ± 0.005 ^{gh}	0.600 ± 0.005 ^{fk}	0.067 ± 0.006 ^a
8	0.665 ± 0.006 ^{abde}	0.646 ± 0.006 ^{bcdg}	0.073 ± 0.006 ^a
9	0.652 ± 0.006 ^{bcfij}	0.644 ± 0.006 ^{bcdg}	0.070 ± 0.005 ^a
10	0.661 ± 0.005 ^{abcde}	0.641 ± 0.005 ^{abcd}	0.069 ± 0.006 ^a
11	0.637 ± 0.007 ^{fgh}	0.624 ± 0.007 ^{ae}	0.067 ± 0.006 ^a
12	0.641 ± 0.006 ^{fgij}	0.612 ± 0.007 ^{ef}	0.066 ± 0.007 ^a
13	0.676 ± 0.005 ^{ak}	0.644 ± 0.005 ^{bcdg}	0.071 ± 0.006 ^a
14	0.651 ± 0.006 ^{bcfij}	0.660 ± 0.006 ^{bghij}	0.082 ± 0.006 ^a
15	0.656 ± 0.005 ^{bcdeij}	0.648 ± 0.005 ^{bcgh}	0.071 ± 0.005 ^a
CO	0.690 ± 0.006 ^k	0.651 ± 0.006 ^{bcghi}	0.214 ± 0.007 ^b

R: red; G: green; B: blue. Results expressed as mean ± SD (standard deviation) of triplicates of color intensity for the R, G, and B channels. Values with different lowercase letters in the same column are significantly different ($p < 0.05$) by Tukey's test.

(a)



(b)

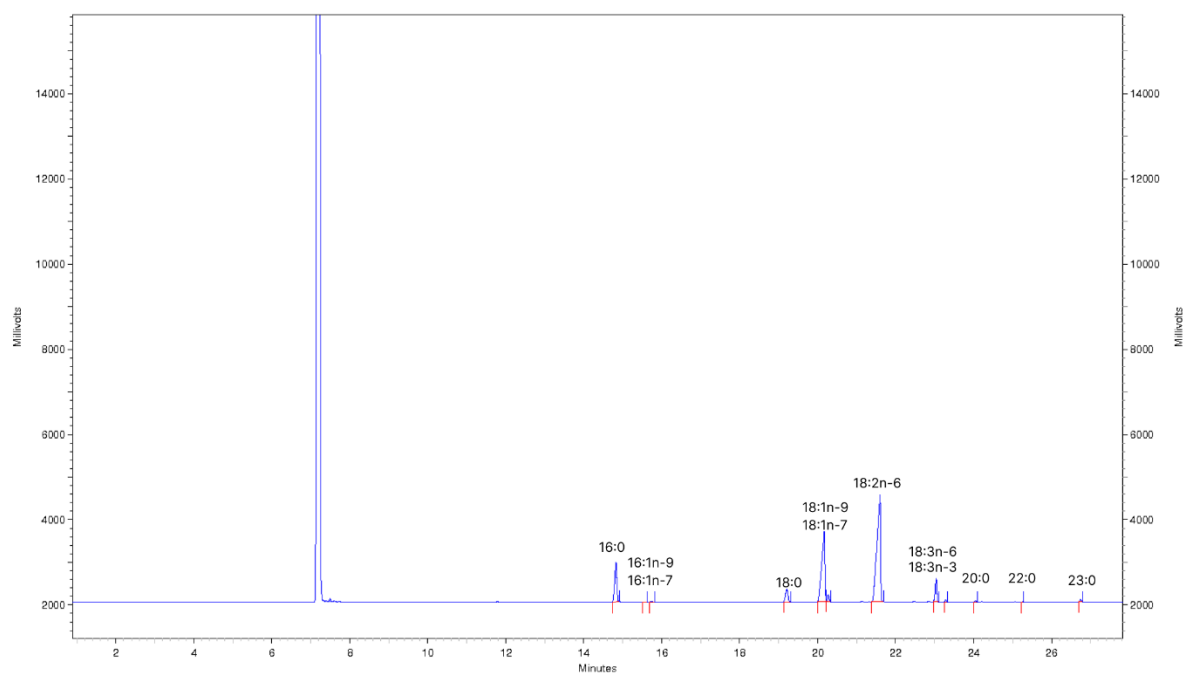


Figure S2. Chromatograms of samples (a) P (b) 14 and (c) SO, obtained by GC-FID.

(c)

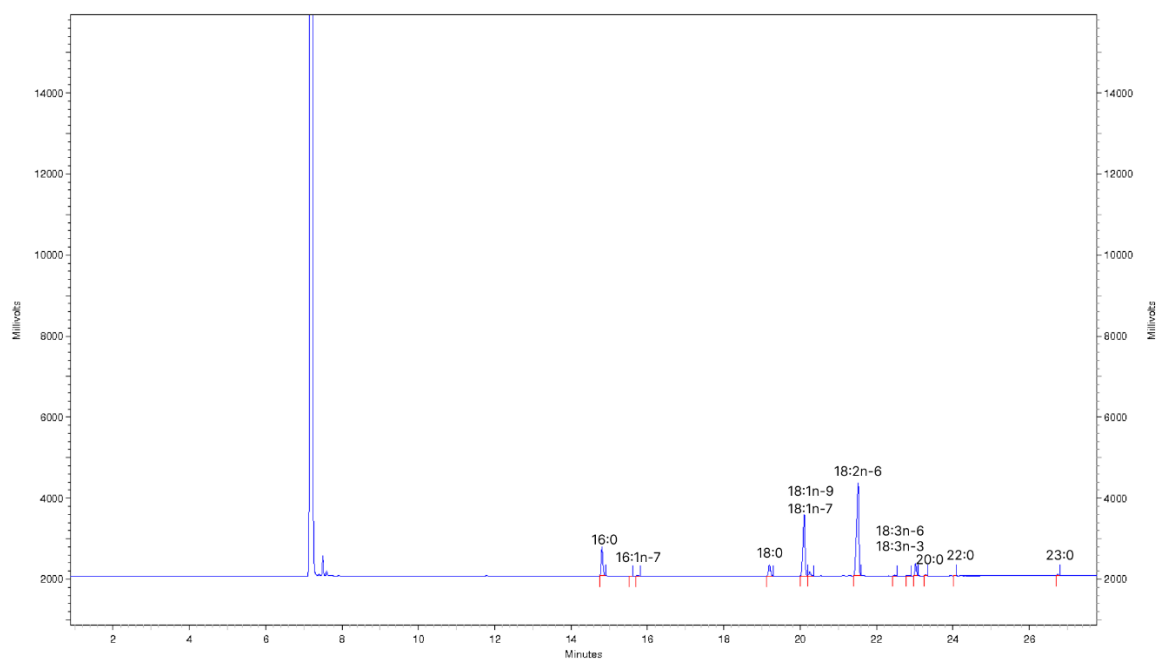


Figure S2. Chromatograms of samples (a) P (b) 14 and (c) SO, obtained by GC-FID (cont.).

Reference

1. International Olive Council; *COI/T.20/Doc. No 33: Determination of Fatty Acid Methyl Esters by Gas Chromatography*; COI: Madrid, 2017. [\[Link\]](#) accessed in March 2025