Supporting information

S1. Procedures

Materials and methods

Six oil samples were purchased from local stores in order to test the method. Three extra virgin olive oils one sunflower and one pomace olive oil were analyzed according to the following analytical methods (*vide infra*). Pyridine 99%, tributyl amine, deuterated chloroform, trifluoroacetic anhydrite 99%, 4-tert-butylphenol 99%, pentan-2-ol 99%, Oleic acid 90%, α-tocopherol 98%, β-sitosterol 70%, cyclohexanol 98%, homovalillyl alcohol 98% 2,2-dimethylcyclohexanol 98%, tyrosol 98%, *tert*-butanol 98%, 1,2-dipalmitoyl-*rac*-glycerol 99%, glyceryl 1,3- dipalmitate 99%, and maleic acid standard for quantitative NMR were purchased from Aldrich.

Preparation of the reagents

Stock solution of trifluoroacetic anhydrite and pyridine solutions, 1.35 M each, were prepared in deuterated chloroform. Teflon needles and glass syringes were used to transfer and measure the volumes of the neat trifluoroacetic anhydrite. Standard solutions of trifluoroacetic anhydrite were kept in glass bottles covered with teflon caps at 4 °C. Closed bottles could be stored for over six months. However routinely used solutions requires replacement with fresh ones every 4 days.

Oleic acid, standards solutions of α-tocopherol, β-sitosterol tyrosol, homovanilyl alcohol, eicosanol, homovanillyl alcohol, 1,2- and 1,3- diglycerols, 2,2'-dimethylcyclohexanol, *tert*-butanol, pentan-2-ol and cyclohexanol and prepared in deuterated chloroform at concentrations of 0.140 M and 10.0 mM. All the solutions were quantified based on the integrals of ^{1}H NMR spectra of the maleic acid analytical standard.

Preparation of the calibration standard solutions

Volumes of 10.0 - 200 μL of the stock solutions of OHs (α-tocopherol, tyrosol, β-sitosterol, eicosanol, homovanillyl alcohol, 1,2- and 1,3- diglycerols, 2,2'-dimethylcyclohexanol and oleic acid) were transferred in the NMR tubes, to give assays with final concentrations 1.40 – 150 mM in OHs. Similar experiments were conducted for the OHs using olive oil as solvent. In each tube, 50.0 μL of the standard (*tert*-butanol or pentan-2-ol or cyclohexanol) were added. Trifluoroacetic anhydrite (50.0 μL 1.35 M) and pyridine (15.0 μL, 1.35 M) were added and the volume was completed up to 500.0 μL with $CDCl_3$. Similar experiments were performed in CD_2Cl_2 for the assignment of the ^{19}F NMR signals originated from the solvents impurities. Precaution must be taken in the use of the trifluoroacetic anhydrite because it is fuming and corrosive and must be handle with care in the hood.

Oil esterification experiments.

No base experiment (Procedure A). An oil sample (0.4000 – 0.5000 g) and internal standard, *tert*-butanol, or pentan-2-ol or cyclohexanol, (50.0 μL, 0.140 M) were allowed to react with excess trifluoroacetic anhydrite (80.0 - 150 μL depending on the H_2O content of oil, 1.35 M in $CDCl_3$) at

room temperature. The mixture was shaken for 1 min, allowed to react for 30 min, and 100 μL of $CDCl_3$ were added. The solution was transferred in a 5 mm NMR tube and measured. The samples were prepared in quadruplicate.

Experiment with base (Procedure B). The same procedure was followed as above with the difference of the addition of base (pyridine or tributyl amine, 25-50 μL, 1.35 M in $CDCl_3$) at room temperature. The reaction is completed in less than 5 min. The samples were prepared in quadruplicate.

Water washing experiments (Procedure C). In this experiment the samples with base were washed several times with a saturated solution of NaCl (5x100 μL). The organic phase was dried with 4 Å molecular sieves before acquisition. The samples were prepared in quadruplicate.

^{19}F NMR. The spectra were recorded using 18.5 μs pulse (90°), an acquisition time of 2.24 s, a spectral width of 2737 Hz, 32 scans (+ 4 dummy scans), delay time 8.0 s, at a 470.4 MHz on a 500 MHz Bruker NMR spectrometer. T1 values calculated from inversion recovery experiments. The chemical shifts are expressed in δ scale (ppm). The region of the NMR studied extends from -76.5 ppm to -74.5 ppm. The calibration for ^{19}F NMR signals was done using $CFCl_3$ as reference.

Quantification procedure and data analysis

The ^{19}F NMR spectra were manipulated with an NMR software [MestreLab Mnova] and a fitting procedure was applied in order to eliminate the line broadening originated from the fast chemical exchange between the anhydrite and the acid trifluoroacetic moieties at the presence of pyridine. The fitting of the spectra resulted in easier and more accurate quantification of the species in solution. The baseline correction and the phase of the spectra was fixed with the same software and the results expressed at mg of compound per Kg of oil ($mg.Kg^{-1}$). The amount of each oil component was calculated as the average of the values from four measurements. LOD and LOQ values were calculated for the OHs from (i) the standard deviation (std) of the intercept and the slope of the calibration curves and (ii) from the S/N ratio calculated for the olive oil spectra and the spectra of blank solutions missing the analyte. All spectra used for LOD and LOQ calculations were derived from 32 scans accumulation.

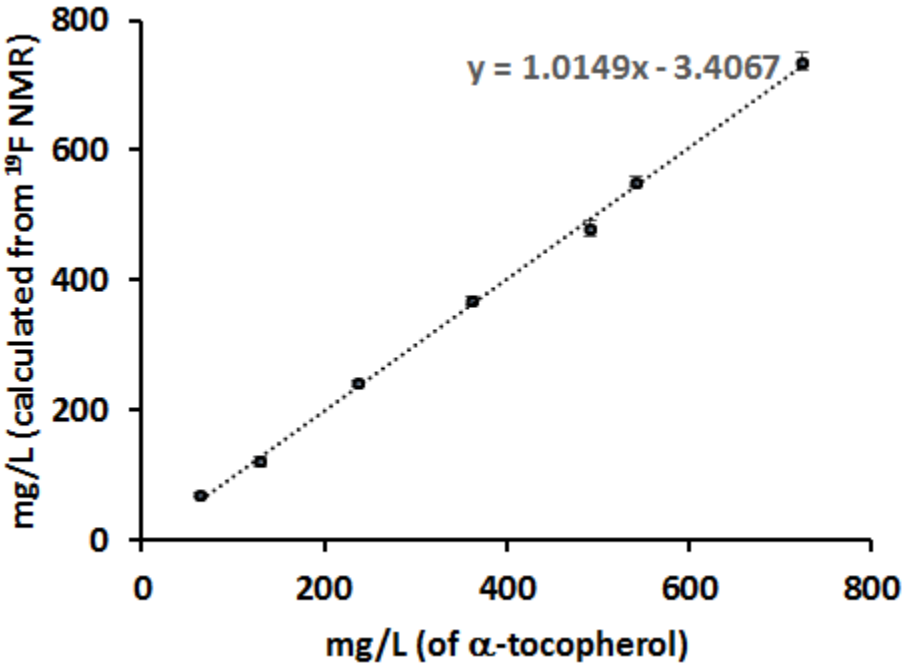

Figure S1. Linear relationship of the added α-tocopherol in $CDCl_3$ *vs* the calculated from the integration of the ^{19}F NMR peak of the α-tocopheryl-trifluoroacetate compared to that of the internal standard (2-pentyl-trifluoroacetate). The purity of the commercial α-tocopherol (95.73 %) used in this experiment was determined by ^{1}H NMR and elemental analysis. The concentration of the added α-tocopherol was accordingly corrected. Experiments performed according to the procedure for the calibration standard solutions. LOD and LOQ were 16 and 49 mg/L respectively based on std calculations. Error bars are too small to see.

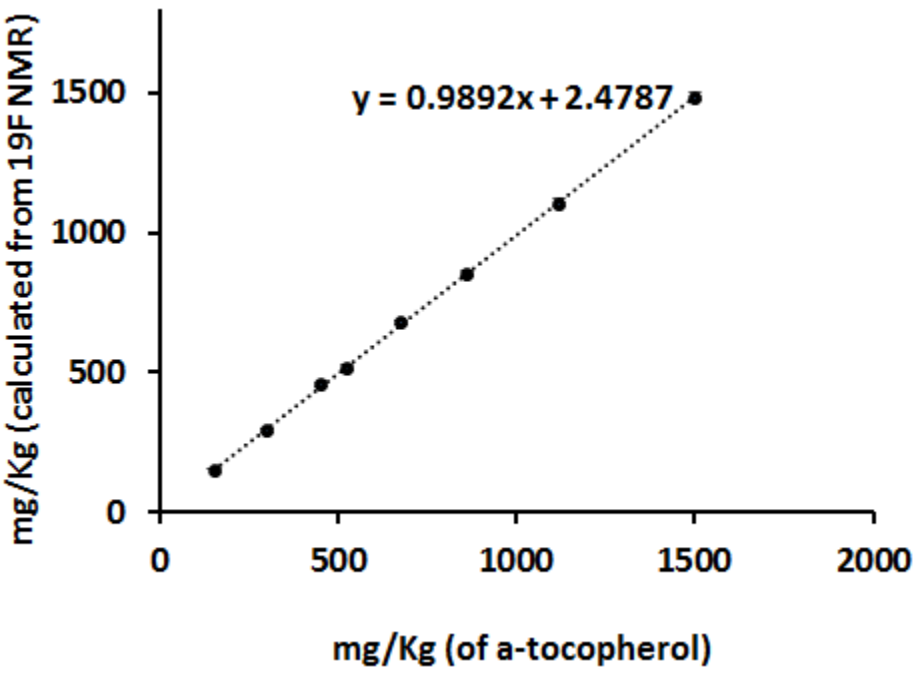

Figure S2. Linear relationship of the concentration of the α-tocopherol (the added plus the natural occurring in the olive oil) *vs* the calculated from the integration of the ^{19}F NMR peaks of the internal standard (2-pentyl-trifluoroacetate) and the α-tocopheryl-trifluoroacetate. The purity of the commercial α-tocopherol (95.73 %) used in this experiment was measured by ^{1}H NMR and elemental analysis. The concentration of the added α-tocopherol was accordingly corrected. Experiments performed according to the procedure **B**. LOD and LOQ were 16 and 47 mg/Kg respectively based on std calculations. Error bars are too small to see.

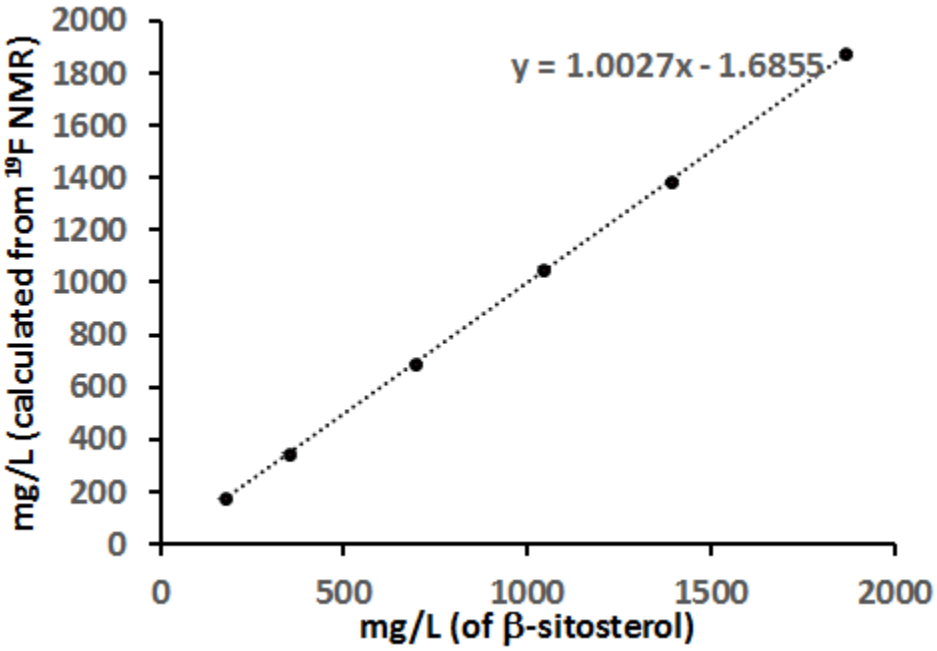

Figure S3. Linear relationship of the added β-sitosterol in $CDCl_3$ *vs* the calculated from the integration of the ^{19}F NMR peaks of the β-sitosteryl-trifluoroacetate compared to that of the internal standard (2-pentyl-trifluoroacetate). The purity of the commercial β-sitosterol (78.45 %) used in this experiment was measured by ^{1}H NMR and elemental analysis. The concentration of the added β-sitosterol was accordingly corrected. Experiments performed according to procedure for the for the calibration standard solutions. LOD and LOQ were 18 and 53 mg/L respectively based on std calculations. Error bars are too small to see.

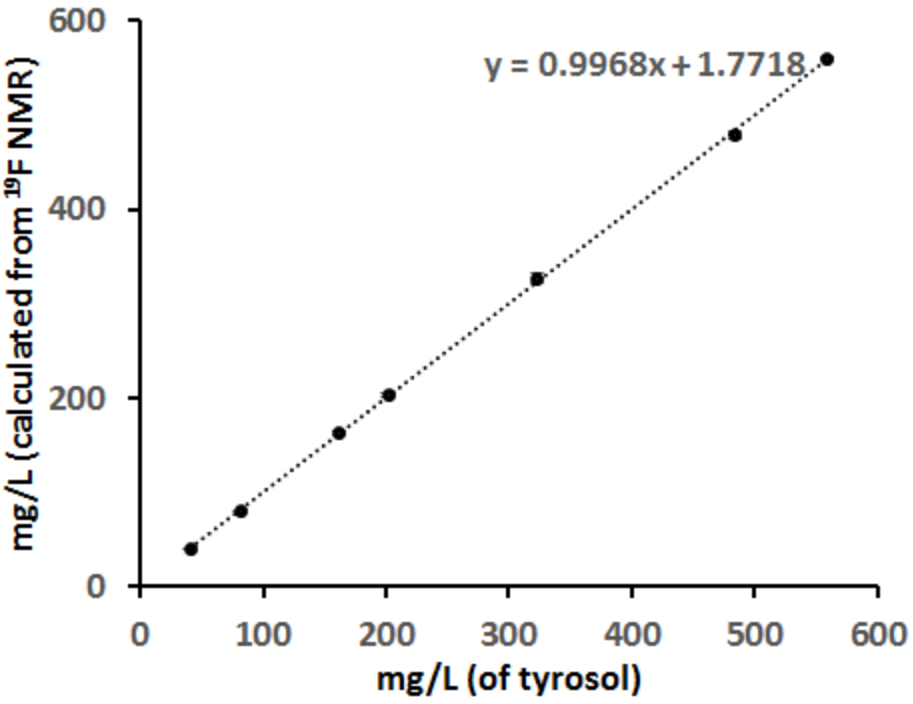

Figure S4. Linear relationship of the added tyrosol in $CDCl_3$ *vs* the calculated from the integration of the ^{19}F NMR peaks of the tyrosyl-trifluoroacetate compared to that of the internal standard (2-pentyl-trifluoroacetate). The purity of the commercial tyrosol (98.12 %) used in this experiment was measured by ^{1}H NMR and elemental analysis. The concentration of the added tyrosol was accordingly corrected. Experiments performed according to procedure for the calibration standard solutions. LOD and LOQ were 7.0 and 22 mg/L respectively based on std calculations. Error bars are too small too see.

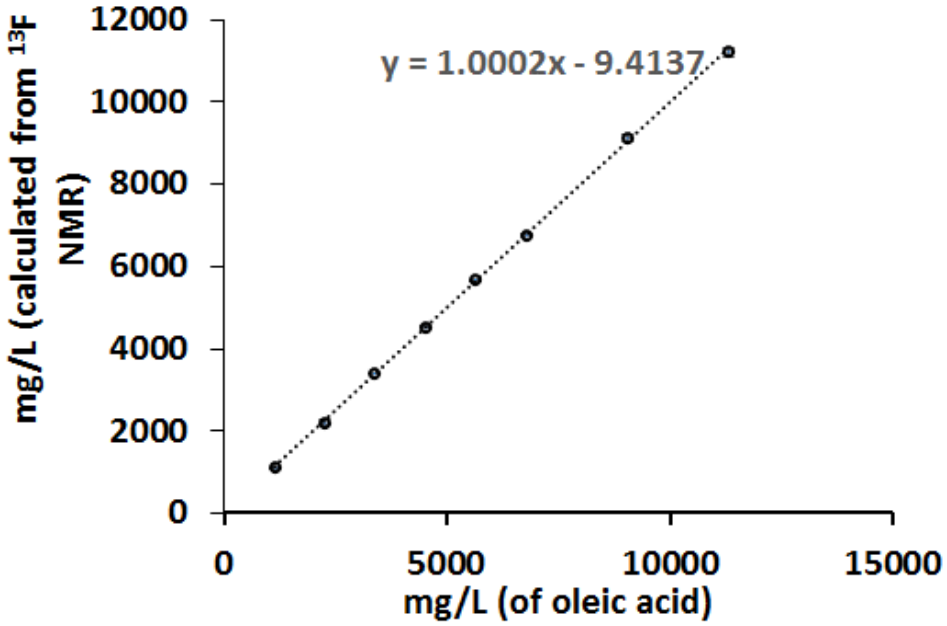

Figure S5. Linear relationship of the added oleic acid in $CDCl_3$ *vs* the calculated from the integration of the ^{19}F NMR peaks of the oleic-trifluoroacetic anhydride compared to that of the internal standard (2-pentyl-trifluoroacetate). The purity of the commercial oleic acid (92.02 %) used in this experiment was measured by ^{1}H NMR and elemental analysis. The concentration of the added oleic acid was corrected accordingly. Experiments performed according to procedure for the calibration standard solutions. LOD and LOQ were 141 and 307 mg/L respectively based on std calculations. Error bars are too small to see.

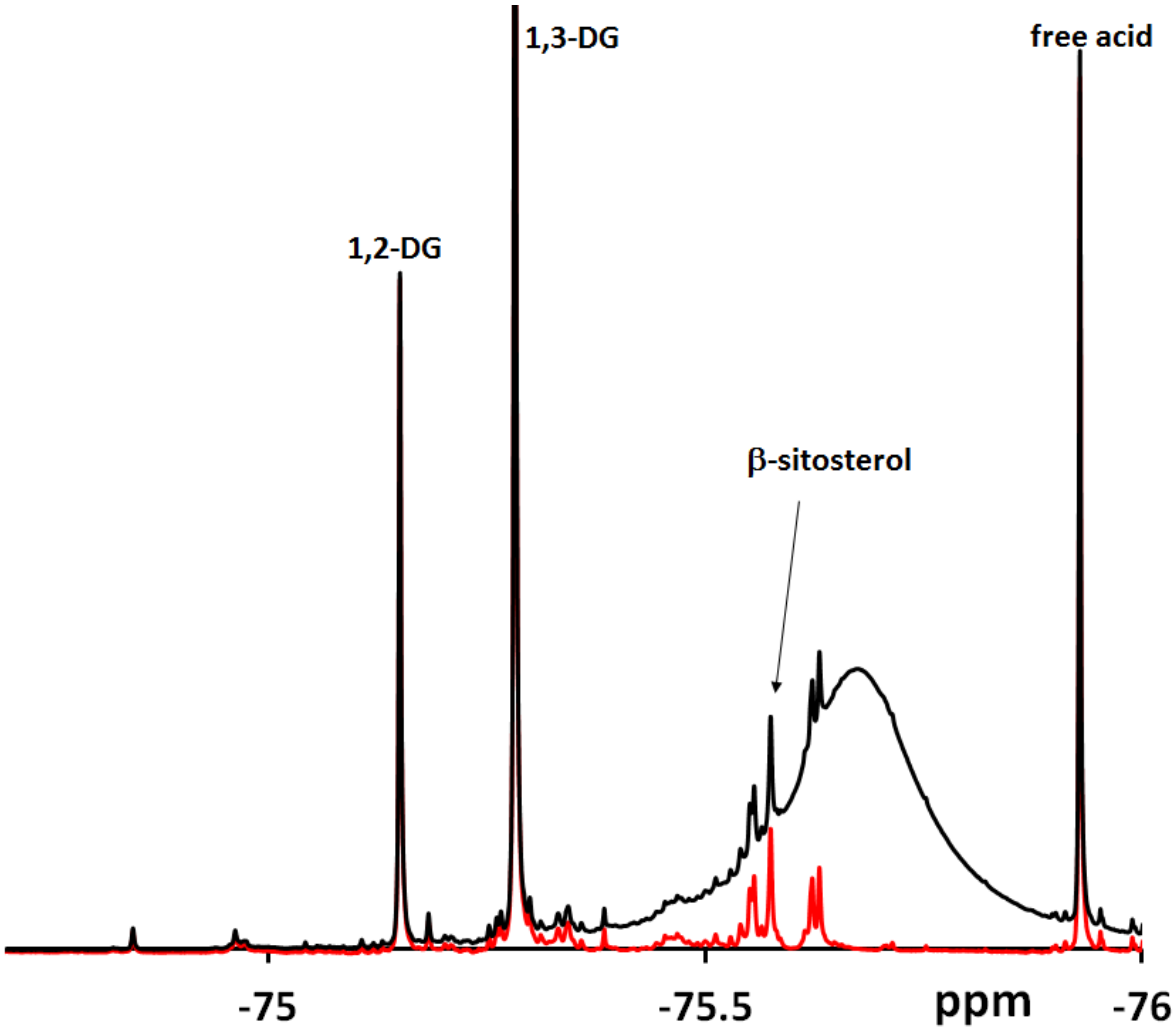

Figure S6. ^{19}F NMR spectrum of virgin olive oil treated with trifluoroacetic anhydrite according to procedure **B** (black line) and the ^{19}F NMR spectrum after subtraction of the broad trifluoroacetic acid/trifluoroacetic anhydrite coalescent peak (red line). The broad trifluoroacetic acid/trifluoroacetic anhydrite peak was substracted by fitting the peak at -75.7005 ppm of 84.45 Hz width and Lorentzian/Gaussian ratio equal to 0.80. DG=diglycerides.

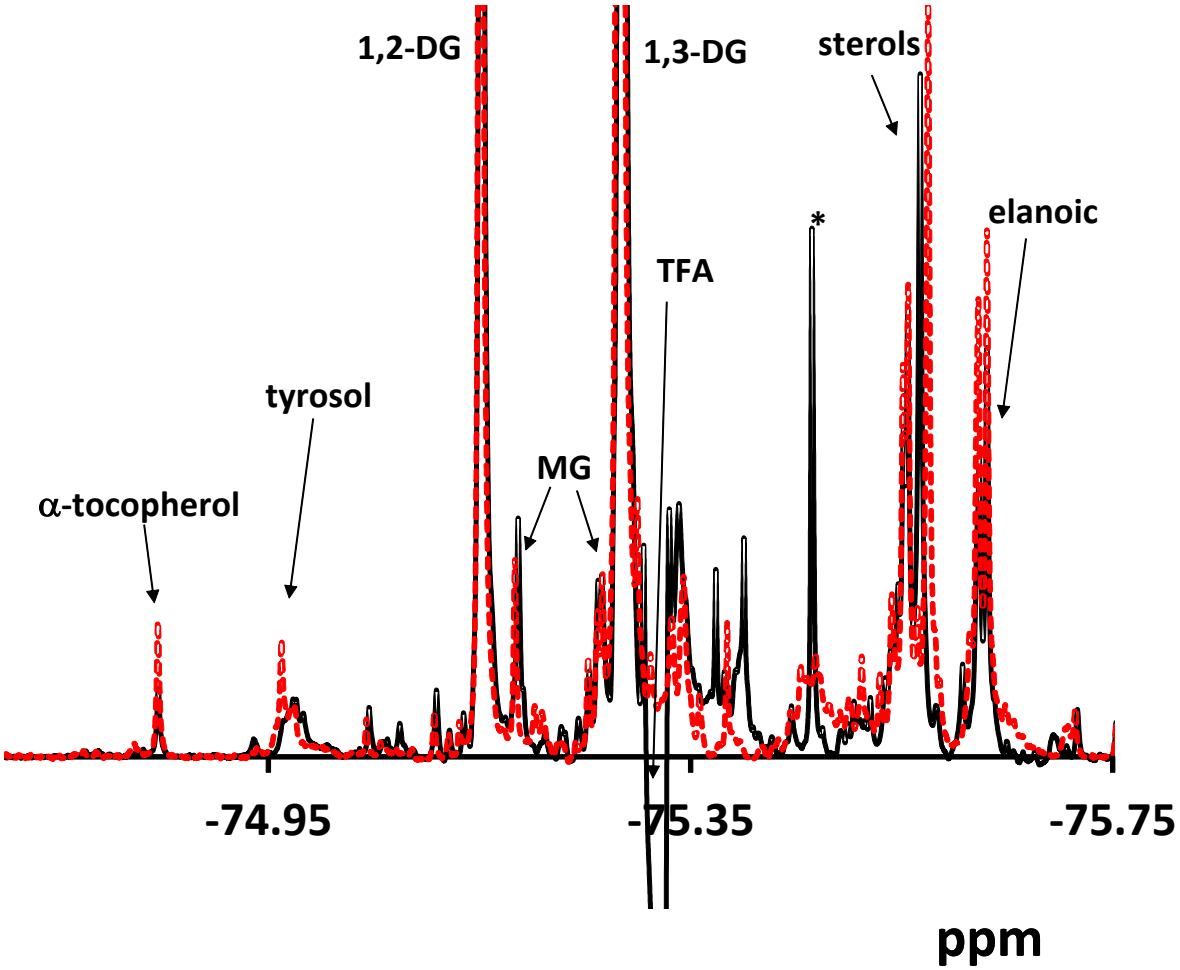

Figure S7. ^{19}F NMR spectrum of a virgin olive oil: treated with trifluoroacetic anhydrite according to procedure **A** (black line) and after the addition of pyridine according to procedure **B,** (red dashed line). In both ^{19}F NMR spectra the trifluoroacetic acid/trifluoroacetic anhydrite peaks have been substracted. The * indicates an impurity contained in the solvent. DG=diglycerides, MG=monoglycerides, TFA=trifluoroacetic anhydrite.

Table S1. ^{19}F NMR chemical shifts of the major CF_3COO-derivatives of the OHs of an oil sample. T1 values calculated from inversion recovery experiments. The sample contained oil (0.457g), *tert*-butanol (50 μL, 0.1400 M in $CDCl_3$), trifluoroacetic anhydrite (100 μL, 1.35 M in $CDCl_3$), pyridine (30 μL, 1.35 M in $CDCl_3$) and 100 μL $CDCl_3$.

OHs Compounds	Chemical Shift / ppm	T1 / s
α-tocopherol	-74.847	0.72
Tyrosol (aromatic)	-74.965	
1,2-DG	-75.150	0.87
1-MG	-75.185	
1,3-DG	-75.264	0.87
β-sitosterol	-75.562	1.66
aliphatic alcohols	-75.31 to -75.50	
other alcohols	-75.558, -75.554	
free fatty acids	-75.929	1.30
allyl, aromatic acids	-75.632, -75.623	

Table S2. The score of four different independent measurements of α-tocopherol in three different edible oils using ^{19}F NMR according to the procedure **B** and HPLC analysis of α-tocopherol of the same oils. (HPLC analysis was performed by *Medalion Labs*, Method of analysis: Samples are saponified by refluxing in ethanolic KOH. The organic extracts are diluted to standard volumes and are submitted to reverse-phase HPLC analysis using a C8 column with isocratic elution. α-tocopherol is detected using a fluorescence detector. Quantitation was performed by comparison to a standard curve.

	mg/Kg of α-tocopherol		
Sample / Anal. Method	**Pomace Oil**	**Virgin Oil**	**Sunflower Oil**
^{19}F NMR			
measurement 1	214	113	594
measurement 2	216	113	603
measurement 3	215	110	590
measurement 4	217	109	592
Mean Value	216±2	111±2	595±5
HPLC measurement	208	111	589

Table S3. The mean values of four different independent measurements of β-sitosterol, 1,2-diglycerides and 1,3-diglycerides in three different edible oils, by ^{19}F NMR according to the procedure **A, B** and **C**, and by GC analysis performed by *Food Alergen Laboratory* (values in parentheses).

	β-sitosterol*	**1,2-DG**	**1,3-DG**
	mg/Kg	**% of all diglycerides**	
Pomace Oil	2396±8 (2375)	36.2 (36.3)	63.8 (63.7)
Virgin Oil	1212±5 (1199)	29.9 (29.0)	70.1 (71.0)
Sunflower Oil	3249±9 (3201)	29.4 (28.2)	70.6 (71.8)

* The amount of β-sitosterol is actually the mixture of β-sitosterol and other minor sterols with similar -OH chemical environment such as campesterol etc. These values are the sum of sterols presents in these oils.