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Better solutions to protect olive oil quality and authenticity

A simplified SPE-GC-FID method for detection of adulteration of olive oil with sunflower oil

Prof. Paolo Lucci, Udine/IT, E. Valli, Bologna/IT, A. Bendini, Bologna/IT, W. Moreda, Sevilla/ES, S. Moret, Udine/IT, T. Gallina Toschi, Bologna/IT

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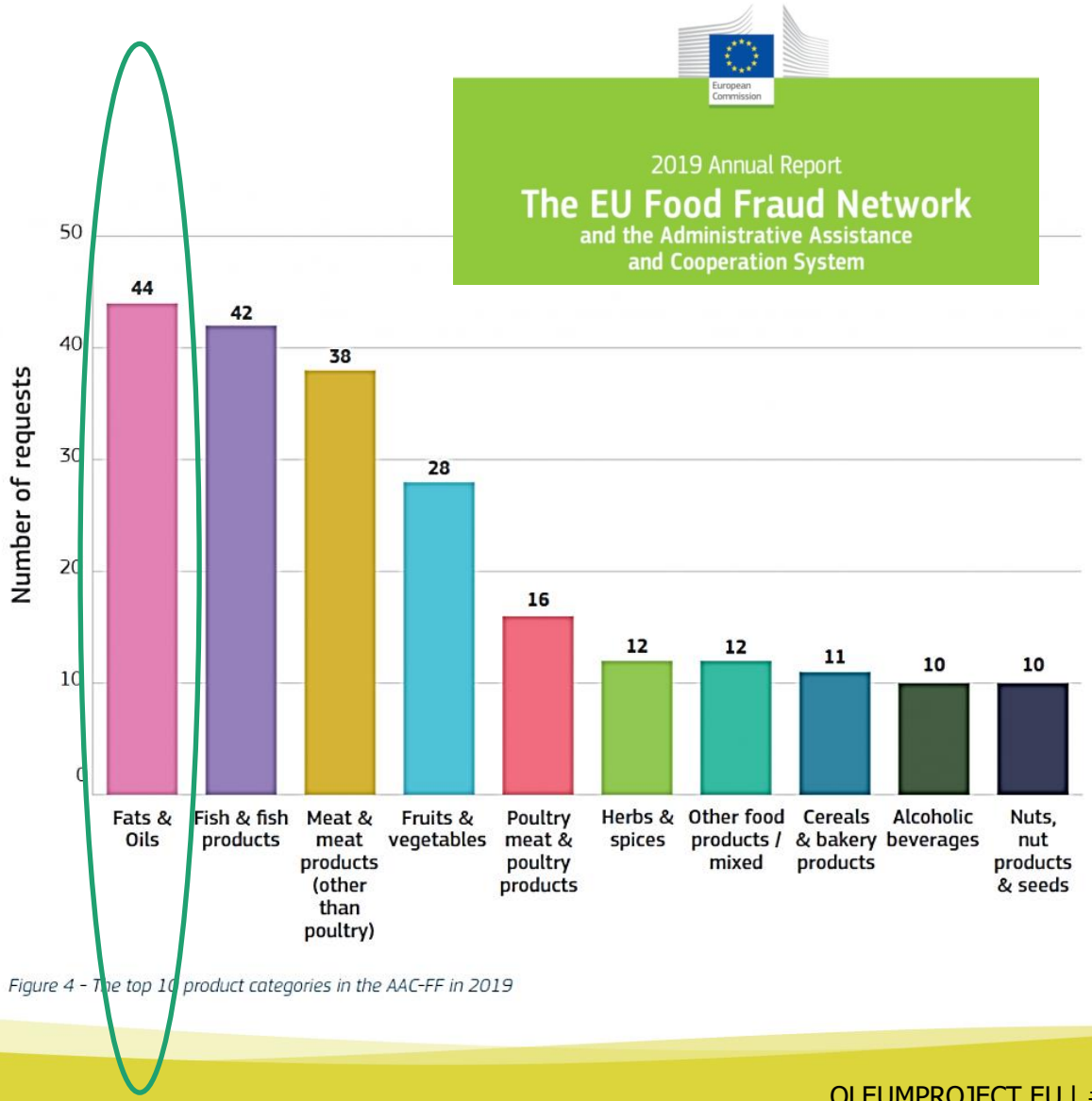

Figure 4 - The top 10 product categories in the AAC-FF in 2019

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- FAs composition
- ΔECN 42
- Sterols composition
- Sterenes
- FA 2n-TAGs
- Waxes
- FAs trans
- UV

Purity criteria

- Free Acidity
- Peroxides Value
- Panel test

Quality criteria

Desmethylsterol composition (% total sterols)

- Cholesterol	≤ 0.5
- Brassicasterol	≤ 0.1
- Campesterol	≤ 4.0
- Stigmasterol	< campesterol in edible oils
- Delta-7-stigmastenol	≤ 0.5
- Apparent beta-sitosterol: beta-sitosterol + delta-5-avenasterol + delta-5-23-stigmastadienol + clerosterol + sitostanol + delta 5-24-stigmastadienol	≥ 93.0

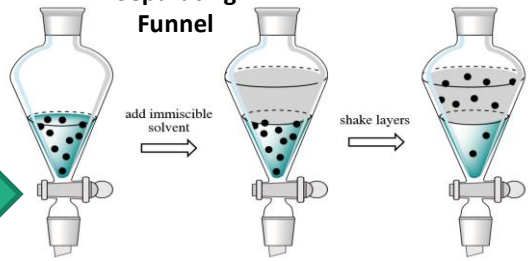
Total sterol content (mg/kg)

Virgin olive oils Refined olive oil Olive oil (ROO+VOOs)	≥ 1000

IOC/T.20/Doc. No 26/Rev.3 June 2018

Flask

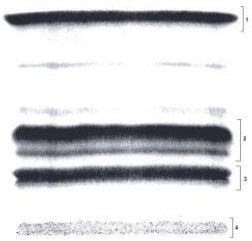
5g Oil
50mL KOH MeOH 2N
60min boiling



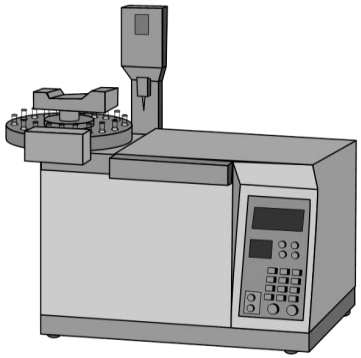
Wash 3 times with ethyl ether
80+70+60mL

100mL
Hexane: Ether 65:35
40/45min

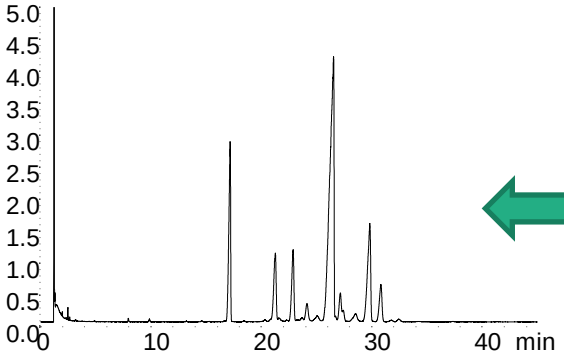
Developing chamber



Extraction and silanization (30min)



45min



Time (5h) - solvent (350mL)

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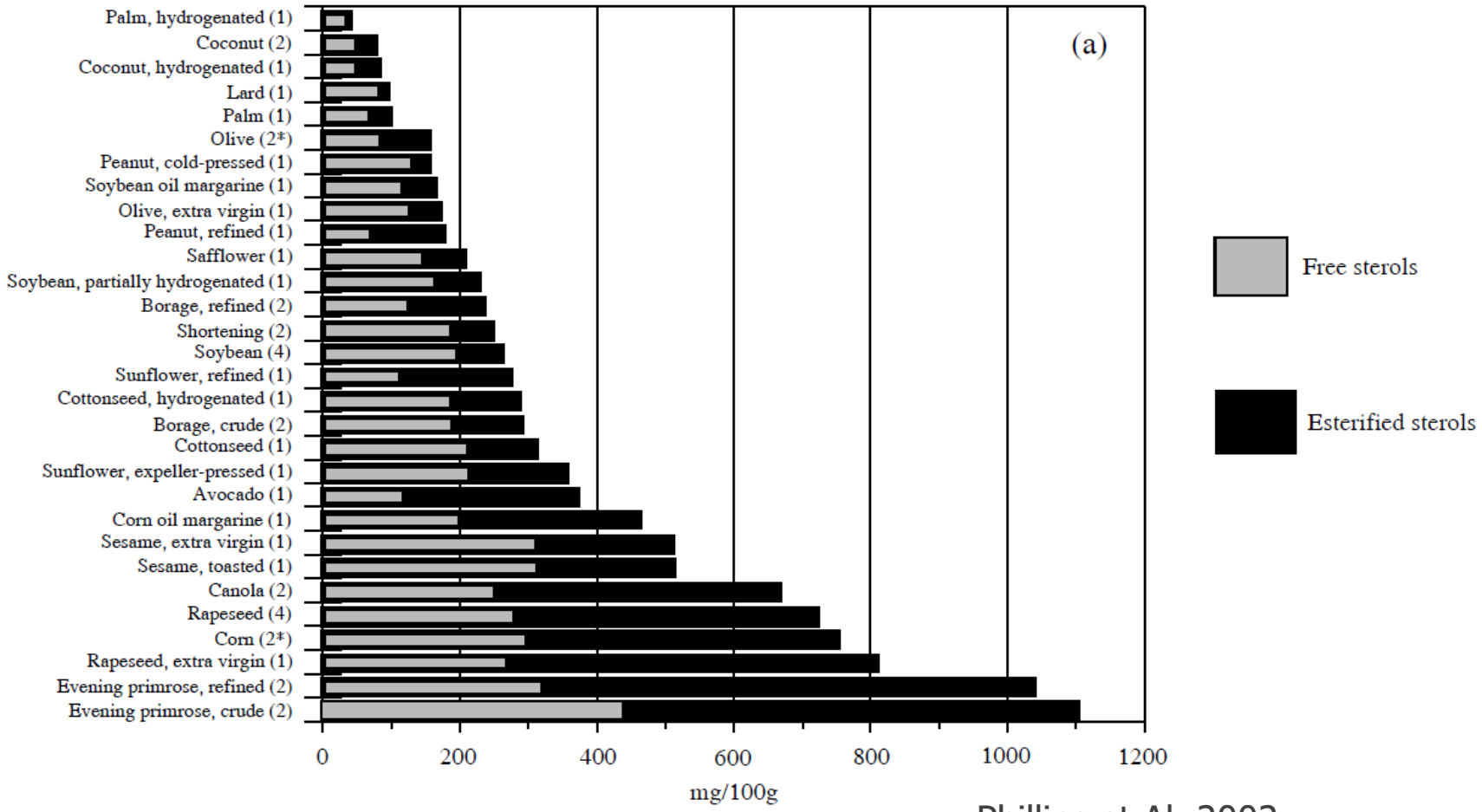
Free and esterified sterols distribution in different vegetable oils



Phillips et Al. 2002

Development of an easy and "fast" method for the identification of both free and esterified minor compounds, including sterols

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Grob K. et Al. 1989

Sample derivatization with pivalic anhydride and heated at 150°C for 15min

↓

diluted 100 times with heptane

↓

20µL Analyzed by LC-GC on-line C_6/CH_2Cl_2 80:20 with 0,05 ACN (45min)

Mariani C. 2010

Sample silanization (20min)

↓

Glass column 40cm, 10mm i.d.
3g silica gel
30ml C6 conditioning
+6mL C6 to remove n-alcane
+60mL C6/$(C_2H_5)_2O$ 99:1 elution

↓

Recovered 1-0,5mL heptane

↓

1µL On-column GC analyses 45/50min

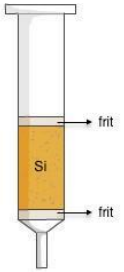
SPME-GC-FID method for free and esterified minor compounds

50mg sample

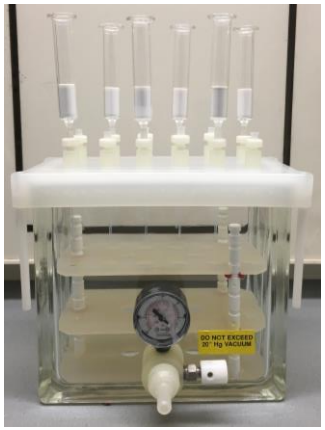
2 I.S.
α-cholestanol
cholesteryl palmitate

+

BSTFA (1% TMS)

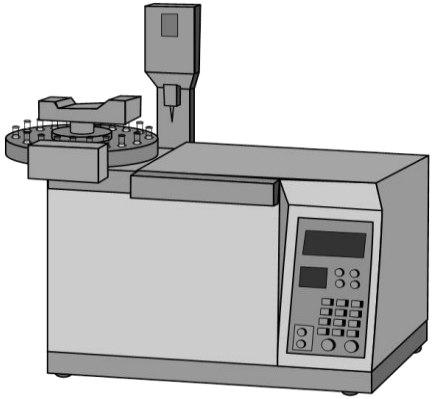


Glass SPE Cartridge
1g Si (60 Å, 0.04–0.015 µm)

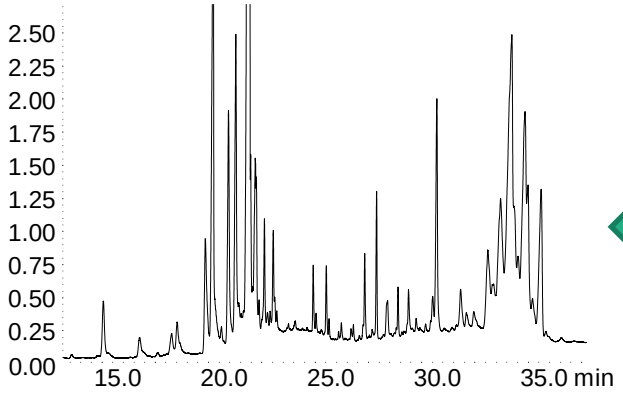


Free and esterified sterols purificarion

10min



35/40min



Time 1.5h & 25mL solvent

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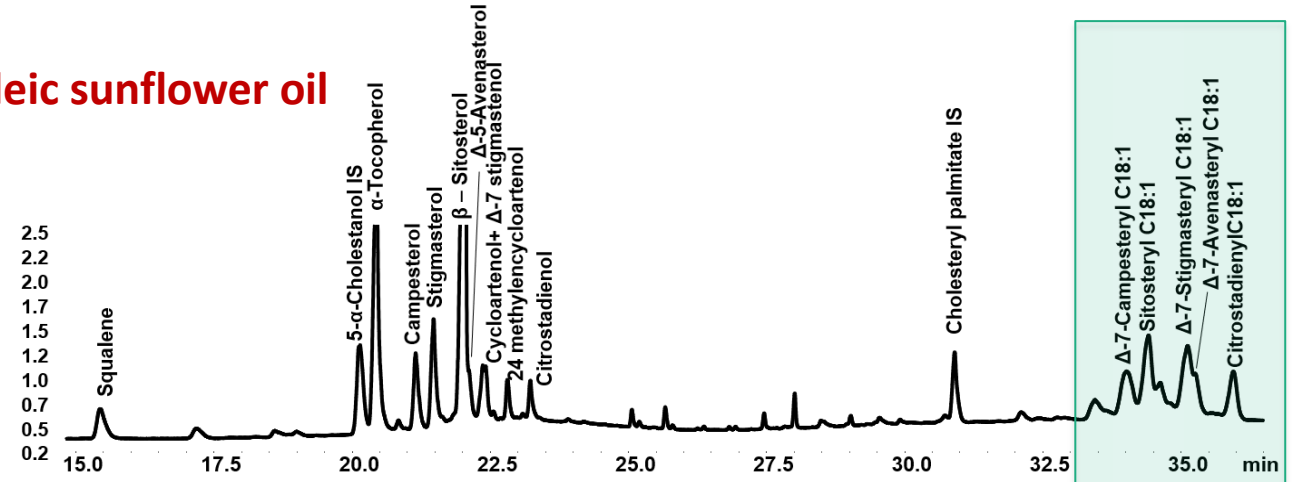
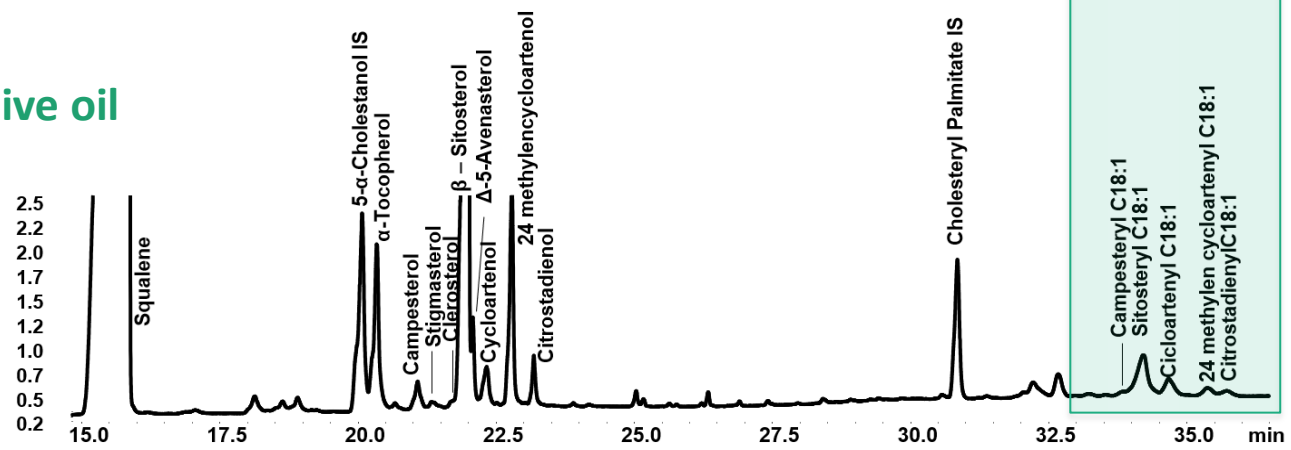
GC chromatograms for free and esterified minor components

Refined high-oleic sunflower oil



Extra virgin olive oil



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In-house validation

Repeatability values for the optimized SPE-GC-FID method

Sample	Free Minor Compounds* (mg/kg)	RSDr (%)	Esterified Minor Compounds$^{\overline{T}}$ (mg/kg)	RSDr (%)
Extra virgin olive oil	1840 ± 110	5.9	820 ± 40	4.6
Refined olive pomace oil	2130 ± 120	5.9	1400 ± 100	7.2
Refined sunflower oil	2400 ± 100	4.2	2460 ± 180	7.5

Results are expressed as average of free and esterified HMC content (mg/kg) of 6 replicates ± repeatability standard deviation. RSDr = repeatability relative standard deviation.

**Sum of resolved/unresolved peaks of cholesterol, brassicasterol, campesterol, stigmasterol, delta-7-stigmastenol, clerosterol, beta-sitosterol, delta-5-avenasterol, cycloartenol, 24-methylencycloartenol, delta-5-23-stigmastadienol, sitostanol, delta-5-24-stigmastadienol, citrostadienol*

$^{\overline{T}}$Sum of resolved/unresolved peaks of campesteryl C18:1, sitosteryl C18:1, cicloartenyl C18:1, delta-7-stigmasteryl C18, delta-7-avenasteryl C18, 24 methylen cycloartenyl C18:1, citrostadienyl C18:1

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Real Samples

Range

Free 1679-2388 mg/Kg

Esterified 357-974 mg/Kg

Range

Free 2278-3030 mg/Kg

Esterified 2682-2798 mg/Kg

		Minor Compounds			
Sample		Free * (mg/kg)	Esterified ∓ (mg/kg)	Total (mg/kg)	Free/Esterified Ratio
Extra virgin olive oils	1a-SP	1970	770	2740	2.6
	2a-SP	1860	460	2320	4.0
	3a-GR	2390	970	3360	2.5
	4a-GR	2090	460	2550	4.5
	5a-GR	1680	360	2040	4.7
	6a-IT	2000	470	2470	4.3
	7a-GR	2040	470	2510	4.3
	8a-GR	2030	560	2590	4.0
	9a-GR	1940	490	2430	3.9
	10a-GR	1980	780	2760	2.5
	11a-GR	1920	490	2410	3.9
	12a-SP	2320	770	3090	3.0
	13a-IT	1790	460	2250	3.9
	14a-PR	1980	900	2880	2.2
	15a-PR	2210	770	2980	2.9
Sunflower oils	1b	2320	2980	5300	0.8
	2b	3030	2800	5830	1.1
	3b	2340	2730	5070	0.9
	4b	2530	2700	5230	0.9
	5b	2310	2660	4970	0.9

Results are expressed as average of free and esterified HMC content (mg/kg) of 3 replicates. Country of origin for EVOO samples: SP = Spain; GR = Greece; IT = Italy; PR = Portugal. * Sum of cholesterol, brassicasterol, campesterol, stigmasterol, Δ-7-stigmastenol, clerosterol, β-sitosterol, Δ-5-avenasterol, cycloartenol, 24-methylencycloartenol, Δ-5-23-stigmastadienol, sitostanol, Δ-5-24-stigmastadienol, and citrostadienol. ∓Sum of campesteryl C18:1, sitosteryl C18:1, cicloartenyl C18:1, Δ-7-stigmasteryl C18, Δ-7-avenasteryl C18, 24 methylen cycloartenyl C18:1, and citrostadienyl C18:1.

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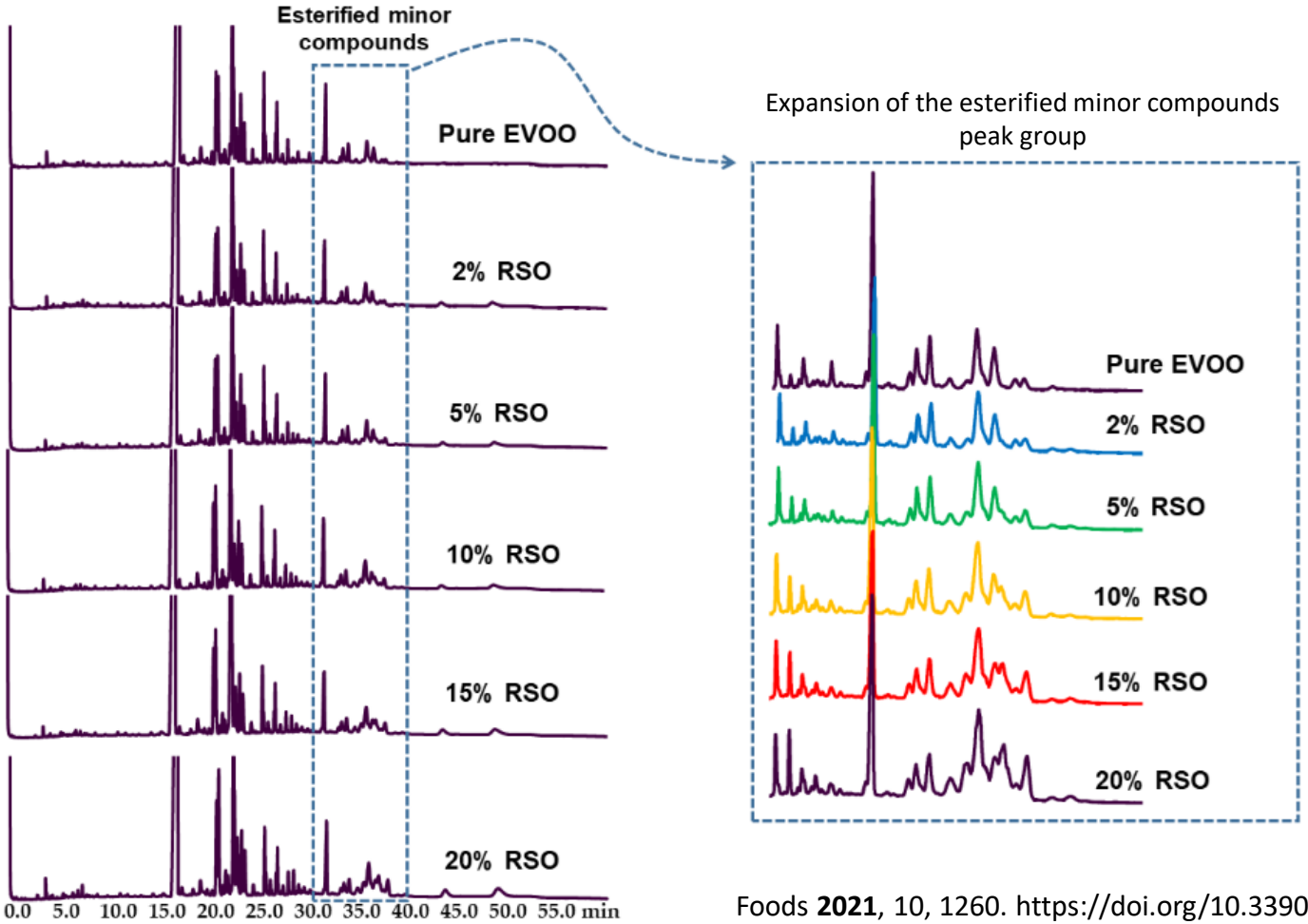
SPE-GC-FID chromatograms of TMS free and esterified HMC profiles of pure EVOO containing different percentages of RSO



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Free and esterified HMC levels of pure EVOO containing different percentages of RSO

		Free minor Compounds *		Esterified Minor Compounds ᵀ		Free/Esterified Ratio
		Theoretical Level (mg/kg)	Experimental Level (mg/kg)	Theoretical Level (mg/kg)	Experimental Level (mg/kg)	
	EVOO		1680		360	4.7
%SO in EVOO	2	1690	1690	410	410	4.1
	5	1710	1760	480	460	3.8
	10	1740	1750	600	630	2.7
	15	1780	1740	720	730	2.4
	20	1820	1730	840	850	2.0

Results are expressed as average of free and esterified HMC content (mg/Kg) of 3 replicates. * Sum of cholesterol, brassicasterol, campesterol, stigmasterol, Δ-7-stigmastenol, clerosterol, β-sitosterol, Δ-5-avenasterol, cycloartenol, 24-methylencycloartenol, Δ-5-23-stigmastadienol, sitostanol, Δ-5-24-stigmastadienol, and citrostadienol. ᵀ Sum of campesteryl C18:1, sitosteryl C18:1, cicloartenyl C18:1, Δ-7-stigmasteryl C18, Δ-7-avenasteryl C18, 24 methylen cycloartenyl C18:1, and citrostadienyl C18:1.

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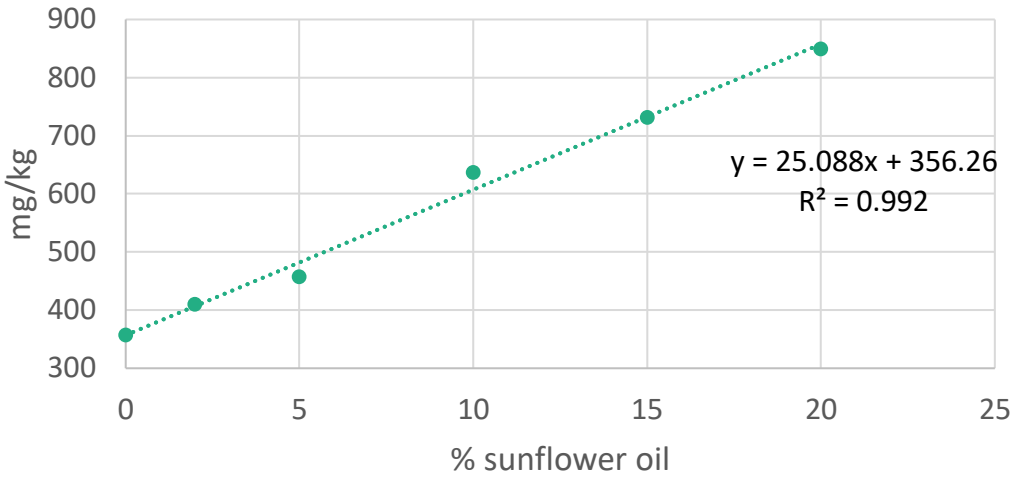
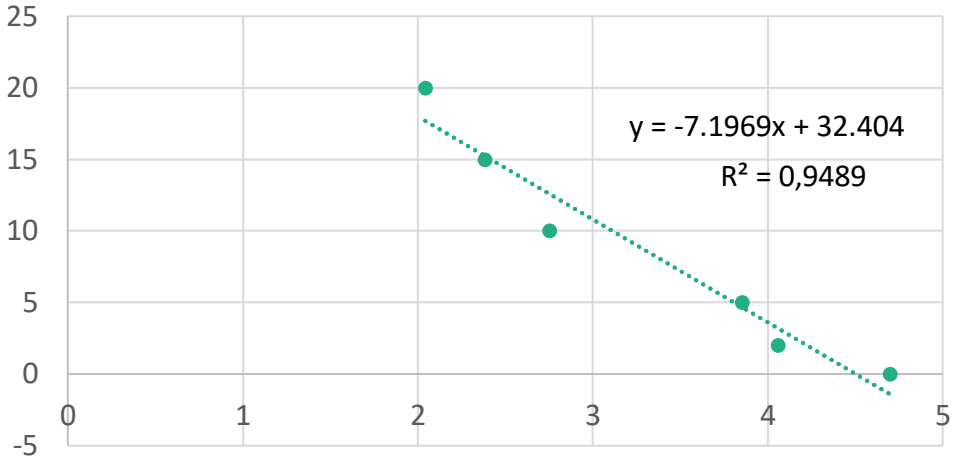
Esterified minor compounds



Real Samples

Blends EVOO - Sunflower

Free/esterified minor compounds ratio



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Free and esterified HMC levels changes during refining process

	Free minor Compounds *	Esterified Minor Compounds ∓	Free/Esterified Ratio
	mg/kg	mg/kg	
Sunflower oil			
Crude	2990	2050	1.4
Bleached	1690	2090	0.8
Deodorized	1470	1920	0.7
Grape seeds oil			
Crude	2710	1260	2.1
Bleached	1460	1250	1.1
Deodorized	1400	1130	1.2
Corn oil			
Crude	5760	5710	1.0
Bleached	3790	5030	0.7
Deodorized	3220	4760	0,6

Results are expressed as average of free and esterified HMC content (mg/Kg) of 3 replicates. * Sum of cholesterol, brassicasterol, campesterol, stigmasterol, Δ-7-stigmastenol, clerosterol, β-sitosterol, Δ-5-avenasterol, cycloartenol, 24-methylencycloartenol, Δ-5-23-stigmastadienol, sitostanol, Δ-5-24-stigmastadienol, and citrostadienol. ∓ Sum of campesteryl C18:1, sitosteryl C18:1, cicloartenyl C18:1, Δ-7-stigmasteryl C18, Δ-7-avenasteryl C18, 24 methylen cycloartenyl C18:1, and citrostadienyl C18:1.

Concluding remarks

The method herein proposed is not intended to replace total sterols method (ISO 12228, COI/T.20/Doc. No. 10). The two methods are completely different from both analytical point of view and final purpose as well the information they may provide.

The two methods could be complementary and used together to obtain a higher degree of information regarding the nature of the oil sample and therefore to reinforce analytical methods available for the prevention of fraud.

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Conclusions

This method determines the free and esterified minor components (free fatty alcohols, free and esterified sterols, free and esterified triterpenic alcohols, sterenes, and free and esterified tocopherols) of olive oils and seed oils and can be utilized as screening tool to detect adulteration by seed oils.

The method proposed in this study has the advantages, compared to the methodologies already proposed in the literature, of minimizing sample manipulation, volume of solvents and reagents, and time needed for the procedure.

In addition, the proposed offline SPE-GC-FID methodology requires the use of simple instrumentation

The method has been successfully in-house validated and showed to be able to detect small increase of esterified HMC due to illegal addition of SFO to EVOO.

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Better solutions to protect olive oil quality and authenticity

A simplified SPE-GC-FID method for detection of adulteration of olive oil with sunflower oil

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