Microwave-assisted saponification and SPE for the simultaneous or alternative analysis of dialkyl ketones and sterols in fats

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Introduction

Sterols and, in particular, phytosterols play an important role in the human diet and in verifying the authenticity of the oil products (e.g., to discriminate olive oil from seed oil). The traditional determination of sterols usually involved a first saponification step, followed by liquid-liquid extraction of the unsaponifiable fraction, TLC for isolation of the sterol and final instrumental determination, most often in gas chromatography (GC) after derivatization. Interesterification is an industrial process used to redistribute the fatty acids among triglycerides changing the physicochemical characteristics of fat (e.g. changing crystallization behaviour and the melting point, improving plasticity). Different from the enzymatic process, the chemical one leads to the formation of dialkyl ketones (DAKs) as by-products. So far, only two papers have dealt with DAKs determination in food [1,2]. Microwave-assisted saponification followed by a lab-made solid-phase extraction (SPE) was optimized for the characterization of either DAK or sterols or both simultaneously. The final instrumental determination was performed by GC-FID for quantification and GC-MS for confirmation purposes

Material and Methods

Microwaves Assisted Saponification (MAS)
2.5 g oil + Iss (14-heptacosanone (C14:0-C14:0) for DAKs α-cholestanol for sterols)
25 mL 2 M (m)ethanolic KOH
MAS settings: 90 °C, 10 min, stirring

Extraction of Unsaponifiable
40 mL, 2 x 30 mL Et_2O
pH neutralization with H_2O

Extract dried and dissolved in
1 mL of C_6

SPE
1 g Silica
Conditioned with 6 mL C_6

Wash (5mL C_6/ Et_2O 98/2 *v/v*)

DAK (5 mL C_6/ Et_2O 96/4 *v/v*)

Wash (25mL C_6/ Et_2O 95/5 *v/v*)

Sterols
(7 mL C_6/ Et_2O 30/70 *v/v*)

***Figure 1**: Scheme of the sample preparation procedure.*

Sterols

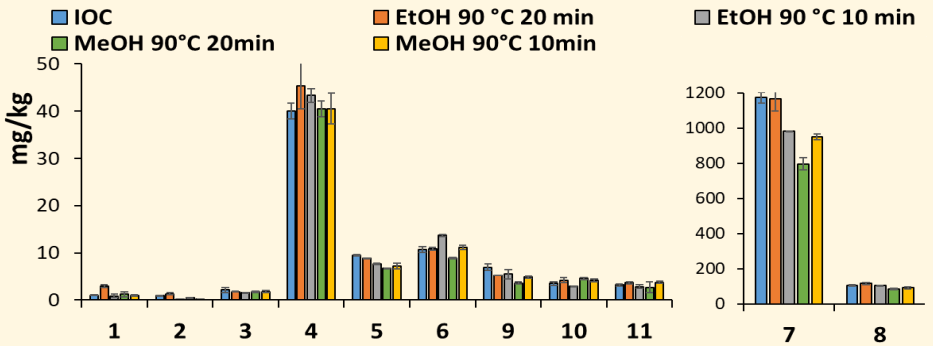

***Figure 2** : Barplot of the sterols from olive oil using the IOC method and the optimized method using MeOH or EtOH for saponification*

***Table 1**: List of targeted sterols*

#	Sterols	#	Sterols
1	Cholesterol	7	β-Sitosterol
2	Brassicasterol	8	Δ-5-Avenasterol
3	24-methylene-cholesterol	9	Δ-5-24-Stigmastadienol
4	Campesterol	10	Δ-7-Stigmastenol
5	Stigmasterol	11	Δ-7-Avenasterol
6	Clerosterol		

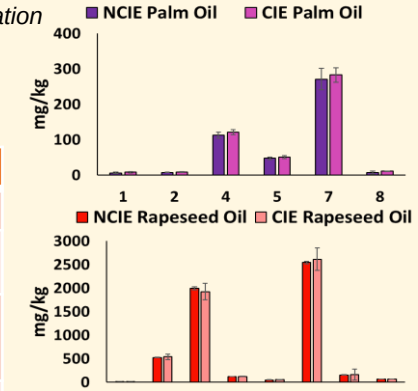

***Figure 3**: Sterols content of no chemically interesterified (NCIE) and chemically interesterified (CIE) of palm oil and rapeseed oil*

DAKs

The DAK profile was studied using GC-MS before quantification with GC-FID. Identification of the different DAKs was not possible based only on the NIST Database; therefore, additional information was obtained by a careful evaluation of the MS fragmentation and by the MS data and elution order. **Figure 4** depicts the profile of the chemically interesterified rapeseed oil along with the MS spectra of all the DAKs detected.

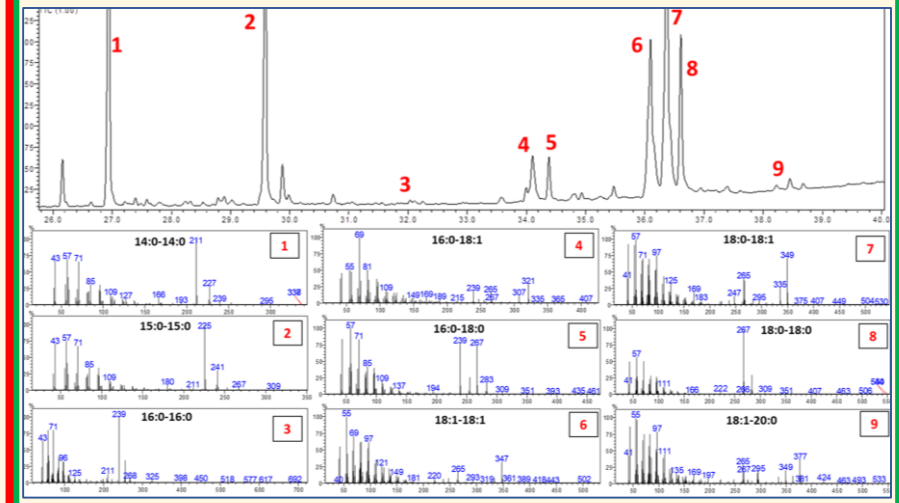

***Figure 4** : GC trace of the chemically interesterified rapeseed oil. The MS spectra of each of the identified peak are reported labeled with the same number of the corresponding peak and reporting the identification of the DAK.*

The proposed method showed good recoveries (>80%) and limit of quantification (0.04-0.07 µg/g for the 4 DAK and of 0.07 µg/g for α-cholestanol). Repeatabilities (n=3) were below 15% for DAKs and generally lower than 6% for sterols. Accuracy on the entire sterol profile was confirmed in comparison to the International Olive Council reference method. The method was finally applied to real-world samples before and after chemical interesterification [3].

References
[1] Mariani, C.; Bellan, G, Riv. Ital. delle Sostanze Grasse 2011, 88, 172–181.
[2] Santoro, V.; Baiocchi, C.; Dal Bello, F.; Gastaldi, D.; Aigotti, R.; Zorzi, M.; Pellegrino, A.; Forte, E.; Romaniello, F.; Magni, M.; et al. , J. Chromatogr. A 2018, 1581–1582, 63–70.
[3] S.Mascrez, S. Danthine, G. Purcaro, Foods, 2021, 10, 445.