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Short communication

Chlorotrimethylsilane, a reagent for the direct quantitative analysis of fats and oils present in vegetable and meat samples

Jordi Eras[a], Javier Ferran[a], Belén Perpiña[a], Ramon Canela[a,b,*]

[a] *Chemistry Department, Lleida University, 25198-Lleida, Spain*
[b] *Centre UdL-IRTA, Lleida University, Rovira Roure 191, 25198-Lleida, Spain*

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Abstract

Acylglycerides present in oil seeds and meat can be transformed into volatile fatty esters using chlorotrimethylsilane (CTMS) and 1-pentanol as reagents. The volatile esters can then be analysed by GC. The method is quantitative and involves only minor sample manipulation. It often permits major recoveries of the total saponifiable lipids present in solid samples. A 40 min reaction time is enough to ensure the total conversion of saponifiable lipids to the corresponding FAPEs.

Keywords: Oil seeds; Chlorotrimethylsilane; Fatty acid pentyl esters

1. Introduction

At present, the method of choice to assess the fatty acid composition of lipids in samples is GLC. Generally, the acylglycerides are extracted from samples and converted to volatile esters. Well-established acid, base and combined catalysis methods are used for this purpose [1,2].

The development of methods for analysing fatty acids that provide rapid and reliable results is currently of great interest. Thus, different methods have been adopted in recent years to remove the extraction step. The derivatization reaction is then performed directly on the sample. These direct methods are called, generically, in situ derivatization [3]. Compared with classical two-step methods, in situ derivatization has several advantages, such as efficiency, simplicity and short processing/reaction time. In these methods, acid catalysts are preferred [4] over base catalysts. This is because base catalysis, reliant on the use of either methanolic solutions of sodium methoxide or other strong alkaline bases, does not allow the esterification of free fatty acids.

Methanolic solutions of H_2SO_4, HCl and BF_3 are the most frequently used for this purpose, but the low solubility of non-polar lipids in the reaction medium [5] entails long reaction times [6] to ensure a complete recovery in quantitative analysis. In order to avoid this problem, the addition of a solvent such as hexane, benzene, toluene or tetrahydrofuran (THF) is recommended [7,8]. It is also possible to use H_2SO_4 in complex mixtures of solvents that include 2,2-dimethoxypropane [8] to accelerate the reaction and to remove water from the samples. Recently, chlorotrimethylsilane (CTMS) has also been used as a catalyst for qualitative in situ fat analyses [9]. The aim of this study is to present a quantitative, in situ method for the assessment of the fatty acid composition of solid samples through their pentyl esters. The reactions are carried out using CTMS and 1-pentanol as reagents and triundecanoin (glyceryl triundecanoate) as an internal standard. The results of the fatty acid analysis, expressed as mg triacylglycerol (TAG)/g of sample, are compared with a two-step, extraction plus derivatization method. Both results are also compared with the content of crude fat in samples obtained by a standard solvent extraction method (EM).

* Corresponding author. Tel.: +34 973 702843; fax: +34 973 238264.
E-mail address: canela@quimica.udl.es (R. Canela).

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2. Experimental

2.1. Chemicals and reagents

Fatty acids were purchased from Sigma (Sigma–Aldrich, Madrid, Spain). 2,6-Di-*t*-butyl-4-methylphenol was obtained from Aldrich (Sigma–Aldrich). Triundecanoin, 1-pentanol and chlorotrimethylsilane were from Fluka (Sigma–Aldrich). Sodium bicarbonate, 35% hydrochloric acid solution, ethanol and diethyl ether were purchased from Prolabo (Prolabo SA, Barcelona, Spain). *n*-Hexane and petroleum ether were from Baker (J.T. Baker, Daventer, Holland). Anhydrous magnesium sulphate was from Panreac (Panreac, Barcelona, Spain).

2.2. Solid and paste samples

Oil seeds and animal tissues were used in the present study. Raw hazelnuts, var. Pauetet, were donated by the Mas Bove Experimental Station (IRTA) in Reus (Spain). Raw sunflower seeds, a slice of medium zone entire *longissimus* muscle of pork and beef, and an entire leg of chicken were purchased from a retail store in Lleida (Spain).

2.3. Sample preparation

The hazelnuts and sunflower seeds were husked and 10 g of each were triturated. Hazelnuts and sunflower samples were stored at −30 °C under nitrogen in polypropylene (PP) boxes. The fat coating of the pork and beef sample slices was removed. Fifty grams of this sample were triturated, freeze–dried, and stored at −30 °C under nitrogen in PP boxes. One hundred grams of the central muscle portion of the pork and beef slice were processed in the same way as the previous samples. The bone and the skin of the chicken leg were removed. The skin and the muscle were processed separately, as were the pork and beef samples.

2.4. Crude fat of seeds and meat samples by solvent extraction

The crude fat content of the samples was obtained using a modification of the AOAC method (AOAC Official Method 991.36, 1998). Three grams of each sample in cellulose thimbles were extracted with petroleum ether–diethyl ether (1:1) mixture using a Det–Gras solvent extraction system (Selecta, Abrera, Spain). The sample was extracted in the boiling stage for 25 min and in the rinsing stage for 30 min. The crude extract was recovered in aluminium cups. The solvent was then removed and the residue was dried for 30 min in an oven at 125 °C. The cups were then cooled and weighed. The assays were carried out in triplicate.

2.5. Preparation of pentyl ester standards

1-Pentanol (2 mL), 2,6-di-*t*-butyl-4-methylphenol (5 mg), the corresponding fatty acid (100 mg) and CTMS (0.5 mL) were added to a 15 mL reaction vial fitted with a PTFE-lined cap. The vial was filled with nitrogen and the mixture was vortex mixed and heated to 90 °C for 4 h in a digestion stirrer block. It was then cooled and 1 mL of 1:1 hexane–diethyl ether mixture was added. The mixture was vortex mixed and neutralized by adding 0.2 g of powdered $NaHCO_3$ and 2 mL of saturated aqueous $NaHCO_3$ solution. The upper phase was recovered and dried over anhydrous $MgSO_4$. The organic extract was filtered and concentrated under vacuum. The residue was purified by column chromatography (silica gel H60, hexane/ethyl acetate) and identified by GLC/MS as the corresponding pentyl ester (fatty acid pentyl ester, FAPE). The purity was established by GLC and TLC.

2.6. Preparation of standard solutions

A standard solution was prepared in a 100 mL volumetric flask by dissolving 0.2 g of triundecanoin in chloroform (2 mg triundecanoin/mL solution). This solution was used immediately after preparation.

A standard pentyl ester solution was prepared by dissolving 0.2 g of each pentyl ester prepared as described above and 0.2 g of pentyl ester of undecanoic acid in 100 mL of chloroform (2 mg FAPE/mL solution). The relative response factors for each fatty acid were calculated by GLC analysis of this solution, as described below.

2.7. Fat/oil extraction prior to derivatization

Samples were extracted using a modification of the AOAC method [10]. Thus, 1 mL aliquots of internal standard solution were added to previously weighed 15 mL reaction vials. The solvent was then evaporated to constant weight using a dry nitrogen stream. Weighed samples, containing about 20 mg of fat, and 5 mg of 2,6-di-*t*-butyl-4-methylphenol, were added to each vial. Next, 0.7 mL of ethanol, previously saturated with nitrogen, and 5 mL of 8.3 M HCl were added to each vial. The vials were filled with nitrogen and fitted with a PTFE-lined cap. The mixture was vortex mixed and heated in a digestion stirrer block at 80 °C for 40 min. Then, 0.7 mL of ethanol was added to each cooled vial. Finally, each vial was extracted three times with a 1:1 hexane–diethyl ether mixture. The extracts were recovered, dried over anhydrous $MgSO_4$, and evaporated to dryness using a nitrogen stream. The recovered oils were weighed and stored at −30 °C under nitrogen in reaction vials.

2.8. Pentyl ester preparation from oils

For each extracted oil, 1-pentanol (2 mL), and CTMS (0.5 mL) were added to a 15 mL reaction vial fitted with a PTFE-lined cap. The vial was filled with nitrogen and the mixture was vortex mixed and heated in a digestion stirrer block at 90 °C for 40 min. The mixture was then cooled and 1 mL of 1:1 hexane–diethyl ether mixture was added. The mixture was vortex mixed and neutralised carefully by adding

0.2 g of powdered $NaHCO_3$ and 2 mL of saturated aqueous $NaHCO_3$ solution (we recommend this step to eliminate any free HCl that has been formed and to ensure capillary GC column durability). The upper phase containing fatty acid pentyl esters was recovered. All the assays were carried out in quintuplicate.

2.9. Pentyl ester preparation from powder and paste samples using the in situ procedure

One milliliter aliquots of internal standard solution were added to previously weighed 15 mL reaction vials. The solvent was then evaporated to constant weight using a dry nitrogen stream. Then, 1-pentanol (2 mL), CTMS (0.5 mL), 2,6-di-*t*-butyl-4-methylphenol (5 mg) and different amounts of each sample were added to each reaction vial equipped with a PTFE-lined cap. Fifty milligrams of powdered hazelnut sample, 50 mg of powdered sunflower sample, 50 mg of pork coating fat, 500 mg of pork muscle, 50 mg of beef coating fat, 500 mg of beef muscle, 70 mg of chicken skin and 500 mg of chicken muscle were used. The vials were filled with nitrogen and the samples were processed as described above. All the derivatization assays were carried out in quintuplicate.

2.10. GC analysis and data record

The samples were analysed in duplicate just after preparation, following injection using a GLC (Trace GC 2000, Thermo Finnigan, Barcelona, Spain) equipped with an FID detector, an electronic pressure control (EPC) system, a split/splitless injection system and an autosampler. The analytical column was a 30 m × 0.25 mm fused silica capillary coated with 0.25 μm film thickness of poly(80% biscyanopropyl–20% cyanopropylphenyl siloxane) (SP-2330) (Supelco, Madrid, Spain). The oven was temperature programmed from 150 °C to 220 °C at 5 °C/min, then held isothermally at 220 °C for 6 min. A 1:20 split injection ratio was used with He as the carrier gas. Volume injection was 1 μL. The injection system and the FID system were held at 270 °C.

Table 1
Fatty acid composition (mg TAG/g sample) ± S.D. is presented for each method and sample indicated

	Raw hazelnuts[a]		Sunflower seeds[a]	
	EDM	ISM	EDM	ISM
C16:0	30.6 ± 2.3	32.1 ± 3.2	28.8 ± 2.8	34.4 ± 2.3*
C16:1	1.2 ± 0.1	1.4 ± 0.1	0.3 ± 0.0	0.3 ± 0.0
C18:0	4.0 ± 0.3	4.5 ± 1.0	16.7 ± 1.7	19.9 ± 1.4*
C18:1	388.4 ± 23.7	413.2 ± 34.9	72.9 ± 7.5	87.8 ± 5.7*
C18:2	80.0 ± 3.9	88.9 ± 6.6*	328.4 ± 33.7	395.5 ± 24.9*
C18:3	1.0 ± 0.1	1.1 ± 0.1	1.5 ± 0.2	1.7 ± 0.1
C20:1	1.6 ± 0.3	2.1 ± 0.3*	0.5 ± 0.1	0.7 ± 0.1*
C22:1			3.4 ± 0.4	4.1 ± 0.2*

[a] $n = 5$.
* This value is significantly different from the corresponding value obtained from the oil-extracted sample ($P < 0.05$).

Table 2
Fatty acid composition (mg TAG/g sample) ± S.D. is presented for each method and sample indicated

	Pork fat[a]		Pork muscle[a]		Beef fat[a]		Beef muscle[a]		Chicken skin[a]		Chicken muscle[a]	
	EDM	ISM	EDM	ISM	EDM	ISM	EDM	ISM	EDM	ISM	EDM	ISM
C14:0	11.9 ± 1.7	11.8 ± 1.0	1.1 ± 0.1	1.2 ± 0.0*	32.7 ± 1.6	34.4±1.2	7.7±0.5	8.0±0.3	8.7±0.5	8.2±1.0	2.9±0.3	2.9±0.2
C16:0	205.7 ± 30.2	218.4 ± 17.7	22.8 ± 1.4	24.3 ± 0.7	250.7 ± 9.6	265.7±8.7	64.6±5.1	68.4±2.5	183.4±10.1	173.9±22.7	62.5±5.3	62.7±3.9
C16:1	19.5 ± 2.6	16.7 ± 1.3	3.3 ± 0.2	3.4 ± 0.1	30.6 ± 1.4	29.8±1.8	8.2±0.5	8.6±0.3	27.9±1.4	26.5±3.4	10.6±1.0	10.5±0.6
C18:0	90.5 ± 14.5	112.9 ± 11.8*	12.5 ± 0.8	12.6 ± 0.5	184.1 ± 4.7	204.8±5.3*	42.9±3.9	46.5±3.3	69.2±3.5	66.6±8.3	26.7±2.3	27.4±1.2
C18:1	404.9 ± 31.2	409.6 ± 31.2	44.4 ± 2.7	45.6 ± 1.5	405.2 ± 12.4	421.7±9.7	112.3±8.9	113.9±3.8	288.3±14.5	278.1±36.9	106.3±9.5	107.7±6.2
C18:2	209.7 ± 33.4	213.5 ± 15.4	12.5 ± 0.8	12.2 ± 0.5	17.8 ± 0.9	17.6±0.5	7.0±0.4	8.4±0.5	175.6±8.5	168.9±22.4	65.9±5.9	66.2±3.9
C18:3	13.7 ± 2.2	14.3 ± 1.1							16.2±0.8	15.7±2.1	5.5±0.5	5.3±0.3
C20:1	9.9 ± 1.7	9.5 ± 0.9	0.9 ± 0.1	0.8 ± 0.0*	2.2 ± 0.1	2.6±0.1*	1.2±0.1	1.5±0.1	2.6±0.1	2.7±0.4		
C22:1	2.6 ± 0.5	2.0 ± 0.4*	0.2 ± 0.0	0.3 ± 0.0*	4.3 ± 0.3	3.2±0.4*	1.4±0.1	1.6±0.1	1.6±0.1	1.5 ± 0.1		

[a] $n = 5$.
* This value is significantly different from the corresponding value obtained from the oil-extracted sample ($P < 0.05$).

Table 3
Weight percentages in sample (% TAG) ± S.D. are presented for each method and sample indicated

	Raw hazelnuts		Sunflower seeds	
	EDM	ISM	EDM	ISM
C16:0	6.0±0.2	5.9±0.1	6.4±0.2	6.3±0.1
C16:1	0.2±0.0	0.2±0.0	0.1±0.0	0.1±0.0
C18:0	0.8±0.1	0.8±0.1	3.7±0.0	3.7±0.1
C18:1	76.6±0.4	76.1±1.0	16.1±0.1	16.1±0.1
C18:2	15.8±0.3	16.4±1.0	72.6±0.1	72.7±0.1
C18:3	0.2±0.0	0.2±0.0	0.3±0.0	0.3±0.1
C20:1	0.3±0.1	0.4±0.0	0.1±0.0	0.1±0.0
C22:1			0.7±0.0	0.7±0.0

FAPE peaks were recorded and integrated using Trace-ThermoQuest computer software.

2.11. Statistical analysis

Individual fatty acid contents were evaluated using the paired *t*-test. Total fat content was evaluated using the Duncan test. Statistical significance was determined at the 95% confidence level. SPSS software (SPSS for Windows 10.1.0) was used to analyse the data.

3. Results

A set of data tables is presented to compare the results from the extraction-derivatization method (EDM), the in situ derivatization method (ISM) and the solvent extraction method for each sample. Tables 1 and 2 show for each fatty acid the mean of its weight expressed as mg TAG_i/g sample and its standard deviation (S.D.). Tables 3 and 4 show the weight percentages and their standard deviations. In addition, Table 5 presents the total lipid weight for each sample and procedure.

4. Discussion

Two different fat-rich seeds and five distinct animal tissues with large differences in their fat contents were chosen to compare the two methods. Pentyl esters showed slightly higher chromatographic retention times than the corresponding methyl esters (fatty acid methyl esters, FAMEs). Nevertheless, no loss of resolution was observed in the chromatograms using the same oven program as for FAMEs [11].

Table 5
Total fat (mg fat/g sample) ± S.D. is presented for each method and sample indicated

Material	*n*	Method (mg fat/g sample)		
		EDM	ISM	EM
Raw hazelnut	5	506.9±25.5 a	543.1±30.9 a,b	554.2±4.5 b
Sunflower seeds	5	452.3±39.9 a	544.3±29.9 b	539.2±29.8 b
Pork fat	5	968.5±129.1 a	1008.8±56.0 a	929.5±3.4 a
Pork muscle	5	97.6±5.2 a	100.4±2.9 a	99.7±1.6 a
Beef fat	5	927.6±21.2 a	979.7±20.6 b	934.5±24.3 a
Beef muscle	5	245.5±13.7 a	253.2±10.5 a	137.0±5.7 b
Chicken skin	5	773.5±39.3 a	742.1±68.6 a	697.6±15.4 a
Chicken muscle	5	280.4±21.4 a	282.64±8.2 a	261.3±3.3 a

Values in the same row presenting the same letter (a, b) are not significantly different ($P < 0.05$).

Although the ISM method usually produces higher values than the EDM method when the weighed amount (mg TAG_i/g sample) of each fatty acid is considered, only sunflower seeds present significant differences between the two studied methods in almost all fatty acids. For the other methods, significant differences in fatty acid composition are only observed in some cases when the two methodologies are compared. Moreover, these differences are randomly distributed among samples and fatty acids.

When weight percentages are considered, the two chromatographic methods produce very close figures (Tables 3 and 4). It is well known that relative percentages avoid problems related with the methods causing incomplete extraction of some fatty acids [4,12]. Nevertheless, fat extraction has to be quantitative when total fat level is needed.

The ISM method seems to be better than the EDM method in fat recovery. Previous studies have also shown that in situ methods tend to give higher fat contents than the other methods [6,12]. Improved efficiency in fat extraction and less fat losses, due to the fact that the in situ method can be considered a one-pot method, could explain these results. It is well known that results depend on factors such as the type of catalysis selected, the solvents used, heating applied dur-

Table 4
Weight percentages in sample (% TAG) ± S.D. are presented for each method and sample indicated

	Pork fat		Pork muscle		Beef fat		Beef muscle		Chicken skin		Chicken muscle	
	EDM	ISM	EDM	ISM	EDM	ISM	EDM	ISM	EDM	ISM	EDM	ISM
C14:0	1.2±0.1	1.2±0.1	1.1±0.0	1.2±0.0	3.5±0.1	3.5±0.1	3.2±0.1	3.1±0.1	1.1±0.0	1.1±0.0	1.0±0.0	1.0±0.0
C16:0	21.3±0.7	21.6±0.4	23.4±0.1	24.2±0.1	27.0±0.2	27.1±0.4	26.3±0.2	26.6±0.4	23.7±0.2	23.4±0.2	22.3±0.1	22.2±0.1
C16:1	2.0±0.1	1.7±0.1	3.3±0.0	3.4±0.1	3.3±0.1	3.0±0.1	3.3±0.1	3.4±0.1	3.6±0.0	3.6±0.0	3.8±0.0	3.7±0.0
C18:0	9.3±0.1	11.2±0.3	12.8±0.0	12.5±0.1	19.8±0.2	20.9±0.2	17.5±0.3	18.1±1.2	8.9±0.1	9.0±0.1	9.5±0.1	9.7±0.1
C18:1	41.8±0.4	40.6±0.3	45.5±0.0	45.5±0.1	43.7±0.2	43.1±0.1	45.8±0.4	44.3±0.6	37.3±0.1	37.5±0.2	37.9±0.1	38.1±0.0
C18:2	21.6±0.1	21.2±0.3	12.8±0.1	12.2±0.1	1.9±0.0	1.8±0.1	2.9±0.1	3.3±0.1	22.7±0.1	22.7±0.0	23.5±0.1	23.4±0.1
C18:3	1.4±0.0	1.4±0.0							2.1±0.0	2.1±0.0	1.9±0.0	1.9±0.0
C20:1	1.0±0.0	0.9±0.0	0.9±0.0	0.8±0.0	0.2±0.0	0.3±0.0	0.5±0.0	0.6±0.1	0.3±0.0	0.4±0.0		
C22:1	0.3±0.0	0.2±0.0	0.2±0.0	0.3±0.0	0.5±0.0	0.3±0.1	0.6±0.0	0.6±0.0	0.2±0.0	0.2±0.0		

ing the synthesis, and the degree of suitability of the procedure chosen for the particular features of each sample [13–15].

The fact that some samples present high standard deviations (S.D.) regardless of the method used could be attributed to the difficulty of obtaining homogeneous samples. The non-uniform distribution of fat depots in samples and the different degrees of their hardness could cause this. This suggestion is supported by the description of similar situations from various authors [3,16].

Significant differences in total lipid weights are found in beef fat and beef muscle samples between the EM and ISM methods. In both cases total lipid weights are higher for the ISM method. The presence of a high fraction of polar lipids could explain these lower figures. Thus, lipids with a high polar fraction may not be efficiently extracted using the Det–Gras solvent extraction system [13,14,17]. However, either acid extraction or CTMS treatment could carry out these extractions and the transformation to volatile esters. In this case, beef muscle would show a high amount of polar lipids that were easily extractable without solvent. The presence of 28% of polar lipids in beef muscle has been reported [16]. Significant differences are also found in beef fat and sunflower seeds when the EDM and ISM methods are compared. Again, in both cases, total lipid weights are higher for the ISM method. As was previously noted, improved efficiency in fat extraction and no fat losses between the extraction and derivatization steps could explain these results.

5. Conclusions

A new in situ quantitative method for analysing fatty acids in samples based on CTMS as catalyst and 1-pentanol as reagent and solvent is proposed. The method entails less sample manipulation and often permits high recoveries of the total saponifiable lipids present in the solid samples. The high solubility of acylglycerides in 1-pentanol ensures this high recovery. No other solvents are required since CTMS and 1-pentanol provide a single reaction phase. A reaction time of 40 min is enough to ensure the total conversion of saponifiable lipids to the corresponding FAPEs.

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