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Short communication

Chlorotrimethylsilane as a reagent for gas chromatographic analysis of fats and oils

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Abstract

Chlorotrimethylsilane can be used as a reagent to transform triglycerides into volatile fatty esters. The volatile esters can then be analysed by GC. The results are fully comparable to those obtained by alternative methods used worldwide. The new one-step method can transesterify acylglycerides and esterify free fatty acids at the same time. Chlorotrimethylsilane is cheaper than $\text{BF}_3\text{-MeOH}$ and is likely to permit the use of different alcohols. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

The characterisation of fats and oils is usually carried out by GC through their fatty acid methyl esters (FAMES) [1]. The triacylglycerides must therefore be transformed into the volatile methyl esters before the analysis.

Although many methods have been developed for carrying out this transformation, they can be divided into three main groups: those using basic catalysis, those using acid catalysis and those that combine the two [2–9]. Potassium hydroxide, sodium hydroxide and sodium methoxide in methanol are the most common catalysts used in basic catalysis, whereas boron trifluoride in methanol ($\text{BF}_3\text{-MeOH}$) is the most common catalyst used in acid catalysis. Potas-

sium hydroxide and boron trifluoride, both in methanol, can be used in combined catalysis, which involves a two-step process. Whereas basic catalysis is faster than acid catalysis, the former will transform free acids into their carboxylic salts and prevent their determination by GC analysis. A second acid catalysis step avoids this problem but increases sample preparation time.

We have already shown that chlorotrimethylsilane (CTMS) used as an acid catalyst allows the transesterification of some fatty esters in 1-propanol [10]. Now we would like to present the ability of these reagents to yield a complete transesterification of triacylglycerides together with the esterification of the free fatty acids that are present.

The results from the developed method are contrasted with those from two other methods that are widely accepted. The first is a two-step method requiring a basic treatment with methanolic KOH

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followed by an acid treatment with 14% boron trifluoride in methanol. The second, like the proposed one, is an one-step acid catalyst method. It also requires the use of 14% boron trifluoride in methanol. The three methods were used to prepare the volatile acyl esters of four different oils and one fat.

2. Experimental

2.1. Fat samples

Olive oil, sunflower oil, palm oil, coconut oil and pork fat were purchased in a retail store and kept under N_2 in refrigerator. They were used without any further purification.

2.2. Chemicals and reagents

CTMS, 1-propanol (PrOH), boron trifluoride in methanol (BF_3 -MeOH) and oleic acid were purchased from Fluka (Sigma-Aldrich, Madrid, Spain). Potassium hydroxide, sodium bicarbonate, acetone, MeOH and hexane were purchased from Prolabo (Barcelona, Spain). The methanol, acetone and hexane were glass-distilled.

2.3. Transesterification of triacylglycerides using CTMS

A 1-ml volume of 20 mg/ml oil or fat propanol solution and 0.5 ml of CTMS was added to a 15-ml reaction vial equipped with a PTFE-lined cap. The mixture was vortex mixed and heated in a shaking water bath at 90°C for 20 min. It was then cooled and neutralised carefully by adding 0.2 g of powdered $NaHCO_3$ and 2 ml of $NaHCO_3$ aqueous saturated solution. The upper phase containing fatty acid propyl esters (FAPREs) was recovered and analysed as described below. The experiments were carried out in quintuplicate.

2.4. Transesterification of triacylglycerides using KOH/ BF_3 -MeOH

Amounts (40 mg) of oil or fat and 1.25 ml of 0.5 M KOH in MeOH were added to a 15-ml reaction vial equipped with a PTFE-lined cap. The mixture

was vortex mixed and heated in a shaking bath at 75°C for 5 min. The vial was then cooled and 2 ml of 14% boron trifluoride in MeOH was added. The mixture was again vortex mixed and heated in a shaking bath at 75°C for 5 min. The vial was cooled and 1 ml of hexane and 1 ml of saturated NaCl solution were added. The mixture was vortex mixed and the upper phase containing the FAMES was recovered and analysed as described below. The experiments were carried out in quintuplicate.

2.5. Transesterification of triacylglycerides using BF_3 -MeOH

Amounts (30 mg) of oil or fat, 1.5 ml of methanol and 1.5 ml of 14% boron trifluoride in MeOH were added to a 15-ml reaction vial equipped with a PTFE-lined cap. The mixture was vortex mixed and heated in a shaking bath at 80°C for 60 min. It was then cooled and 2.5 ml of water and 2.5 ml of hexane were added. The mixture was vortex mixed for 15 min and the upper phase containing the FAMES was recovered and analysed as described below. The experiments were carried out in quintuplicate.

2.6. TLC analysis

The samples were analysed by thin-layer chromatography (TLC) using silica-gel 60 (Merck, Barcelona, Spain) and hexane-acetone-acetic acid (95:5:0.1) as the mobile phase. Oleic acid and the corresponding oil or fat were used as standards.

2.7. GC analysis and data record

The esters were analysed just after preparation in triplicate using a ThermoQuest series 2000 chromatograph equipped with a FID detector, an EEP system (Fisons, Barcelona, Spain), a split/splitless injection system and an autosampler. The analytical column (Supelco, Madrid, Spain) was a 30 m×0.25 mm fused-silica capillary coated with 0.25 μ m film thickness of poly(80% biscyanopropyl-20% cyanopropylphenyl siloxane) (SP-2330), and was temperature-programmed for olive oil, sunflower oil, palm oil and fat pork from 150 to 220°C at 5°C/min, then held at 220°C for 6 min. For coconut oil it was

temperature-programmed from 70 to 220°C at 10°C/min and then held at 220°C for 5 min. A 1:20 split injection ratio was used with He as the carrier gas.

FAME and FAPRE chromatographic peaks were recorded and integrated using TRACE-THERMOQUEST computer software. The data were presented as the percentage obtained by area normalisation.

Correction factors for detector response for the different methyl and propyl esters were used according to Jorgensen et al. [11].

2.8. Statistical analysis

Correlation studies between data were carried out applying the minimum least squares regression method according to Miller and Miller [12]. Confidence limits for the y axis intercept values (a) and the slope values (b) ($P \leq 0.05$) were determined according to the same authors. Microsoft EXCEL 97 software was used to process the data.

3. Results

Chromatographic profiles of FAMES and FAPRES obtained by two of the tested methods corresponding to coconut oil are presented in Fig. 1. TLC analyses showed that neither remaining triacylglycerides or free fatty acids were detectable in samples after the application of any of the three methods.

Table 1 shows the means of areas of methyl and propyl esters obtained from olive oil, sunflower oil, palm oil, coconut oil and pork fat using the three described methods. We studied the similarity between the methods using linear regression. Thus, the individual means obtained by one method were assigned to the x axis, and the respective means of the same oil or fat obtained by another method to the y axis [12]. The regression curve was calculated by minimum least squares. Table 2 shows the y axis intercept value (a), slope (b), standard error ($S_{y/x}$) and determination coefficient (R^2) for each tested sample and pair of methods. Confidence limits ($P \leq 0.05$) for the y axis intercept values ($a_{95\%}$) and the slope values ($b_{95\%}$) together with the number of data point are also indicated in Table 2.

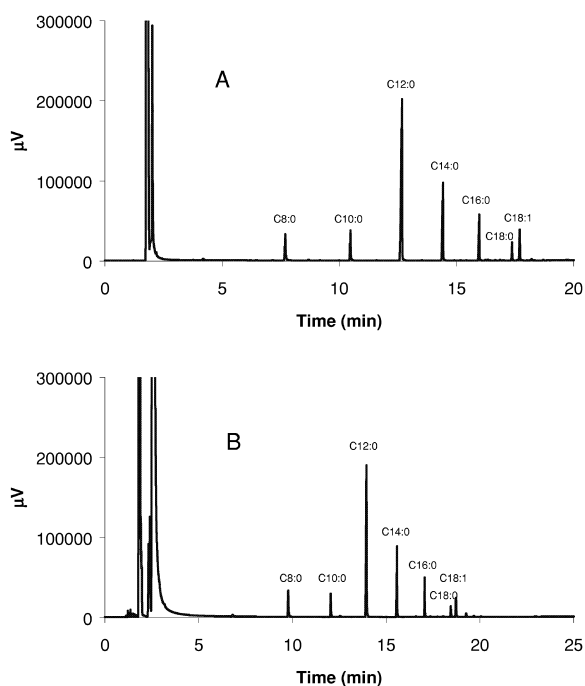


Fig. 1. Separation by GLC of the fatty esters obtained from coconut oil. (A) Methyl esters obtained using KOH/BF₃-MeOH method. (B) Propyl esters obtained using the proposed method. The column was a 30 m × 0.25 mm fused-silica capillary coated with 0.25- μ m film thickness of poly(80% biscyanopropyl-20% cyanopropylphenyl siloxane) (SP-2330), and was temperature-programmed from 70 to 220°C at 10°C/min, then was held at 220°C for 5 min. A split injection ratio 1:20 was used with He as the carrier gas.

4. Discussion

GC time analysis is similar for FAPRES and FAMES using the same oven program and no observable differences in resolution are observed. Although great variations are not observed in the results of the three methods, BF₃-MeOH alone tends to give a higher yield in saturated acids. These results could be a consequence of the method applied. The use of 14% BF₃-MeOH for 1 h could cause a partial degradation of unsaturated acids and, thus, increase the relative amount of saturated acids. Ackman has already proposed to carry out this reaction under N₂ atmosphere using a 3.5% BF₃-MeOH solution to avoid such degradation [7]. The $a_{95\%}$ and $b_{95\%}$ values show in all cases that the results obtained with the proposed method can be

Table 1

Fatty acid composition (%)±standard error are presented for each method and oil or fat assayed. Correction factors for detector response for the different methyl and propyl esters were used according Jorgensen et al. [11]

Method ^a	Olive oil			Sunflower oil			Palm oil			Coconut oil			Pork fat		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
C _{8:0}										5.93±0.39	7.16±0.71	8.99±0.57			
C _{10:0}										5.85±0.16	5.84±0.30	7.70±0.52			
C _{12:0}										50.74±0.25	50.07±0.57	51.37±1.41			
C _{14:0}							1.01±0.01	0.97±0.03	1.24±0.02	19.14±0.14	18.98±0.65	17.03±0.50			
C _{16:0}	11.11±0.12	11.62±0.09	12.23±0.08	7.23±0.08	6.87±0.19	7.52±0.10	47.15±0.20	47.01±0.32	48.19±0.28	9.71±0.12	9.66±0.32	8.02±0.57	27.63±0.20	28.10±0.61	27.53±0.12
C _{16:1}	0.76±0.01	0.79±0.01	0.87±0.02	0.22±0.03	0.19±0.11	1.21±0.13	0.14±0.03	0.14±0.02	0.66±0.12				1.64±0.04	1.59±0.02	1.92±0.34
C _{18:0}	2.72±0.02	2.67±0.01	2.79±0.04	4.22±0.04	4.26±0.09	5.37±0.03	5.39±0.05	5.21±0.05	5.59±0.03	3.09±0.05	2.84±0.34	2.38±0.45	17.43±0.09	17.71±0.35	18.72±0.11
C _{18:1}	78.36±0.08	77.63±0.09	76.62±0.39	31.49±0.08	31.70±0.64	30.98±0.42	38.14±0.13	38.31±0.18	37.58±0.22	5.54±0.25	5.50±0.42	4.51±1.00	38.67±0.22	37.90±0.15	36.56±0.29
C _{18:2}	6.39±0.07	6.34±0.01	6.69±0.06	56.60±0.19	56.93±0.57	54.59±0.43	7.80±0.05	8.05±0.06	6.37±0.04				13.68±0.26	14.04±0.28	14.28±0.03
C _{18:3}	0.61±0.01	0.96±0.01	0.94±0.36	0.23±0.01	0.21±0.05	0.32±0.01	0.36±0.02	0.34±0.01	0.37±0.01				0.9±0.02	0.9±0.01	0.97±0.06

^a A, KOH/BF₃-MeOH method; B, CTMS method; C, BF₃-MeOH method.

Table 2

Comparison of the different assayed methods by linear correlation. The number of data points (n) and the values of slope (b) \pm 95% confidence limit ($b_{95\%}$), y axis intercept (a) \pm 95% confidence limit ($a_{95\%}$), standard error ($S_{y/x}$) and coefficient of determination (R^2) are presented

Material	Methods ^a	n	$b \pm b_{95\%}$	$a \pm a_{95\%}$	$S_{y/x}$	R^2
Olive oil	A vs. B	6	0.9887 \pm 0.01152	0.1986 \pm 0.37377	0.2825	0.9999
Olive oil	B vs. C	6	0.9843 \pm 0.01334	0.2826 \pm 0.42940	0.5339	0.9997
Olive oil	A vs. C	6	0.9732 \pm 0.02177	0.4788 \pm 0.70629	0.3236	0.9999
Sunflower oil	A vs. B	6	1.0077 \pm 0.00978	-0.0992 \pm 0.26097	0.1795	1
Sunflower oil	B vs. C	6	0.9455 \pm 0.02514	0.8819 \pm 0.67412	0.4647	0.9996
Sunflower oil	A vs. C	6	0.9528 \pm 0.0259	0.7878 \pm 0.69104	0.4753	0.9996
Palm oil	A vs. B	7	0.9999 \pm 0.00912	0.0054 \pm 0.21181	0.1718	0.9999
Palm oil	B vs. C	7	1.0072 \pm 0.05376	-0.1070 \pm 1.24761	1.0121	0.9978
Palm oil	A vs. C	7	1.0074 \pm 0.04541	-0.1064 \pm 1.05374	0.8549	0.9985
Coconut oil	A vs. B	7	0.9813 \pm 0.03338	0.2766 \pm 0.70754	0.5381	0.9991
Coconut oil	B vs. C	7	1.0181 \pm 0.11166	-0.2691 \pm 2.34418	1.7675	0.9910
Coconut oil	A vs. C	7	0.9977 \pm 0.13012	0.0329 \pm 2.75815	2.0978	0.9873
Pork fat	A vs. B	6	0.9891 \pm 0.03962	0.2280 \pm 0.84886	0.4702	0.9992
Pork fat	B vs. C	6	0.9621 \pm 0.05596	0.5923 \pm 1.19623	0.6572	0.9983
Pork fat	A vs. C	6	0.9510 \pm 0.08296	0.8224 \pm 1.77731	0.9845	0.9961

^a A, KOH/BF₃-MeOH method; B, CTMS method; C, BF₃-MeOH method.

considered equivalent to those from the two-step method within the confidence levels. Thus, calculated slopes and intercepts do not differ significantly from the 'ideal' values of 1 and 0, respectively [12]. The BF₃-MeOH method does not meet these requested levels in four cases, corresponding to olive and sunflower oil samples. Such discrepancies between methods have already been described by several authors [1,7–9,13].

5. Conclusion

A new one-step method for preparing esters of fatty acids from oil and fats based on acid catalysis is proposed. The method is able to transesterify acylglycerides and esterify free fatty acids at the same time. It is cheaper than using BF₃-MeOH and is likely to permit the use of different alcohols. The possibility of using 1-propanol instead of methanol ensures a major solubility of fat, which results in a short reaction period without a considerable increase in GC analysis time. Moreover, CTMS is easier to handle than either HCl gas or concentrated H₂SO₄,

two alternative acid catalysts, and its solubility in organic solvents ensures one reaction phase. The procedure is simple and time-saving, and the results are comparable to other well-established methods. Research is in progress to assess the possibility of using different alcohols and applying the method directly to solid samples avoiding the oil extraction step.

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