

Profiling fatty acids in vegetable oils by reactive pyrolysis–gas chromatography with dimethyl carbonate and titanium silicate

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Abstract

A novel methodology in on-line pyrolysis–gas chromatography (Py–GC) for the fast analysis of fatty acids in vegetable oils with minimal sample treatment and the use of non-toxic reagents is described. Pyrolysis at 500 °C for 10 s of sub-microgram quantity of vegetable oil dissolved in dimethyl carbonate (DMC) and in the presence of nanopowder titanium silicon oxide resulted in the production of fatty acid methyl esters (FAMES) as unique products. Pyrolysis performed by means of a resistively heated filament pyrolyser interfaced to a GC–MS apparatus enabled the direct analysis of evolved FAMES. The DMC/Py–GC–MS analysis was tested on soybean, coconut, linseed, walnut and olive oil and the results compared to the classical BF₃–methanol as reference methodology. The DMC method exhibited a lower precision and was biased towards lower levels of polyunsaturated fatty acids (PUFA) in comparison to the BF₃–methanol method, but was more advantageous in terms of reduced sample treatment, waste generation and risk factors of employed chemicals.

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1. Introduction

Gas chromatography is often the technique of choice for analysing the composition of fatty acids in lipids [1]. Fatty acids mostly occur as involatile triglycerides in vegetable oils, therefore their conversion into the corresponding methyl ester derivatives (FAMES) is commonly accomplished prior to GC analysis. The production of FAMES from triglycerides requires procedural steps which may include extraction, hydrolysis, methylation, *trans*-esterification, and so forth. Methanolysis with boron trifluoride (BF₃) followed by solvent extraction is a well-established procedure for profiling fatty acids in oils and fats [2]. However, it needs a relatively large amount of solvents and laborious sample manipulation, such as heating under reflux conditions, that hamper automated determinations [3]. More in general, sample preparation is often time consuming and requires toxic or corrosive substances which may pose environmental and health hazard. Reducing the use of dangerous or waste-generating

chemicals is of interest also in the field of analytical methodology, and to this purpose the term green analytical chemistry has been quoted in the literature [4–7]. Although the amount of chemicals employed for lipid analysis is generally low, the overall quantity can be significant for laboratories dealing with elevated sample throughput (e.g. quality control in food industry). In this context, reducing tedious manual work-up and preventing the use of dangerous solvents or reagents is of interest in method development. Pyrolysis-methylation or thermochemolysis with an *in situ trans*-methylating reagent is a solvent-less one-step procedure, as the production of FAMES takes place directly inside a thermal unit directly interfaced to a GC or MS apparatus for on-line detection [8–15]. In recent years, researchers have adopted novel approaches to on-line GC/MS coupling for *in situ trans*-methylation of fatty acids in complex matrices [10–15]. However, pyrolysis methylation is generally accomplished with corrosive *trans*-methylating reagents, such as tetramethylammonium hydroxide (TMAH) and trimethylsulphonium hydroxide (TMSH). Dimethyl carbonate (DMC) can be used as an alternative *trans*-methylating reagent for the production of FAMES under pyrolytic conditions [16]. DMC is reputed a green reagent in organic synthesis with several advantages, as it is neutral,

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odourless, cheap, non-corrosive, non-toxic, and exhibits good solvent properties [17–19]. In order to act as a methylating reagent, DMC requires the use of a catalyst. In our previous work, we found that zeolite 13X promoted the formation of FAMES as predominant products, but solely under off-line pyrolysis conditions [16].

In this study, we show that when vegetable oil and excess DMC are pyrolysed in the presence of a commercially available titanium silicate, FAMES are formed as unique products under on-line Py–GC conditions. The performance of the procedure for profiling fatty acids is discussed by comparison with the popular BF_3 –MeOH method.

2. Experimental

2.1. Materials

Soybean and coconut oils were purchased from Lardan Chemicals, linseed and walnut oil from Kremer, olive oil from Remilia. Dimethyl carbonate (DMC), BF_3 (50% in methanol), fatty acids methyl ester standard mixture (palmitic, stearic, oleic, linoleic and linolenic) and titanium silicon oxide (99.8%, nanopowder) were from Sigma–Aldrich. The following abbreviations were used for FAMES: 8:0, octanoic; 10:0, decanoic; 12:0, dodecanoic; 14:0, tetradecanoic; 16:0, hexadecanoic (palmitic); 18:0, octadecanoic (stearic); 18:1, (*Z*)-9-octadecenoic (oleic); 18:2, (*Z,Z*)-9,12-octadecadienoic (linoleic); 18:3, (*Z,Z,Z*)-9,12,15-octadecatrienoic (linolenic); acid methyl esters.

2.2. DMC procedure

Oils were dissolved in DMC to a 1% (w/w) final concentration. An aliquot (10 μL) of the oil/DMC solution was spread into 5 mg of nanopowder titanium silicate held at the central part of a quartz tube sample holder by quartz wool. The mixture was pyrolysed at 500 °C for 10 s at the maximum heating rate using a CDS 1000 pyroprobe heated platinum filament pyrolyser (Chemical Data System, Oxford, USA) directly connected to the injection port of a Varian 3400 gas chromatograph coupled to a Saturn 2000 ion trap mass spectrometer (Varian, Walnut Creek, CA, USA).

Preliminary experiments performed at different pyrolysis temperatures in the 400–800 °C range resulted in similar GC–MS profiles. The effect of pyrolysis time was not investigated, but presumably the *trans*-esterification reaction took place rapidly prior to the fast volatilisation of DMC. Pyrolysis temperature and time were set at 500 °C and 10 s, respectively, to conform with published works on TMAH-thermochemolysis with the same pyrolytic apparatus [14].

2.3. BF_3 –methanol procedure

Oils were analysed following the AOAC-IUPAC official method with slight modifications [2]. About 150 mg of oil were refluxed for 10 min with 4 mL methanolic NaOH 0.5 M. Then, 5 mL of methanolic BF_3 were added and the mixture refluxed for

2 min followed by the addition of 10 mL of *n*-hexane. After cooling a saturated NaCl aqueous solution was added under stirring, then the hexane layer containing FAMES was separated, dried over anhydrous sodium sulphate and diluted prior to GC–MS analysis.

2.4. GC–MS analysis

A Supelco Omegawax capillary column (30 m \times 0.25 mm i.d., 0.25 μm film thickness, non-bonded poly(ethylene glycol) as stationary phase) was used with a temperature programme from 120 to 220 °C (held for 10 min) at 10 °C min^{-1} with helium as carrier gas. For the analysis of coconut oil, the thermal programme was from 50 to 220 °C (held for 10 min) at 10 °C min^{-1} . The Varian 1078 programmable split/splitless injector (split mode) was maintained at 250 °C and the Py–GC interface at 250 °C. Mass spectra were recorded at 1 scan s^{-1} under electron ionisation at 70 eV, scan range 45–650 m/z . Structural assignment of the products was based on match with the NIST 1992 mass spectra library and GC–MS injection of pure compounds.

FAME distribution was expressed as percentage peak area determined in the total ion chromatograms and reported as mean \pm standard deviation (SD) from at least three replicate analyses.

3. Results and discussion

3.1. Qualitative aspects

On-line pyrolysis of soybean oil in the presence of dimethylcarbonate (DMC) and nanopowder titanium silicate produces the GC–MS profile exemplified in Fig. 1A. It is evident from the GC–MS results that the only reaction products are fatty acid methyl esters (FAMES). Underivatized fatty acids and hydrocarbons, which are typically formed upon pyrolysis without any added reagent [16,20], are not detected. In our previous study, we observed that FAMES could be obtained only as minor products when pyrolysis of soybean oil with DMC was performed in the presence of zeolite 13X [16]. In that case, the low yield of FAMES was attributed to the fast volatilisation of DMC from the hot Py–GC interface which prevented the methylation of pyrolytically formed fatty acids. Here, the stronger efficiency of nanopowder titanium silicate in trapping DMC and promoting its methylating activity are probably responsible for the rapid formation of FAMES.

The chromatogram obtained from DMC/Py–GC–MS is rather similar to that deriving from the BF_3 –MeOH procedure (Fig. 1B). However, besides the main peaks associated to the typical fatty acids of soybean oil (i.e. palmitic, stearic, linoleic and linolenic acids), the DMC-derived pyrogram contains smaller peaks which are absent or negligible in the chromatogram from the BF_3 –MeOH procedure. According to the GC–MS characteristics, these additional peaks are assigned to structural isomers of methylated linoleic acids. The catalytic properties of titanium silicon oxides are possibly responsible for the thermal isomerisation of double bonds [21].

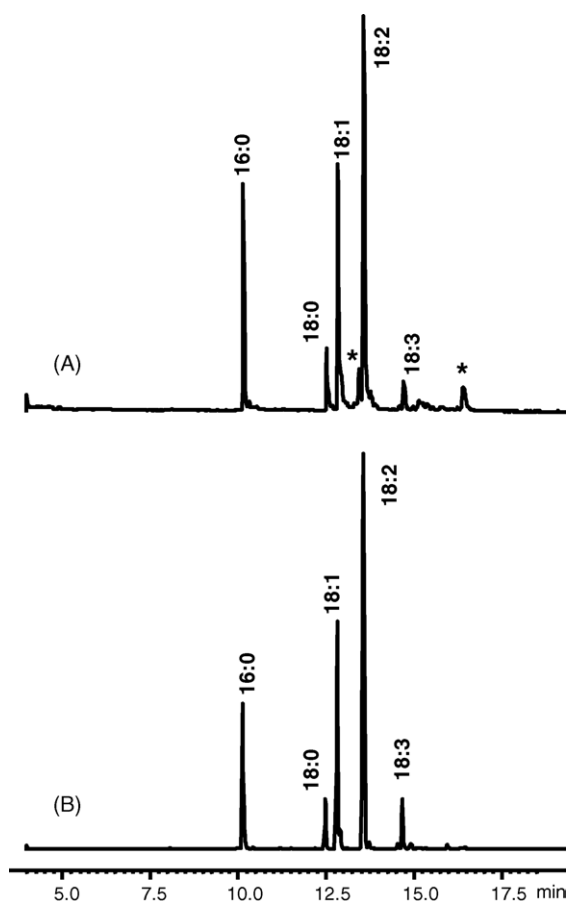


Fig. 1. Total ion chromatogram obtained from (A) DMC/titanium silicate/Py-GC-MS and (B) BF_3 -methanol analysis of soybean oil. (*) isomers of linoleic acid methyl ester.

The DMC/Py-GC-MS method was applied to a suite of vegetable oils, namely olive, linseed and walnut, which are characterised by the same fatty acids found in soybean oil. Typical chromatograms are collectively shown in Fig. 2. As in the case of soybean oil, FAMES typical of the analysed oils are the principal compounds, but lower levels of isomers of PUFAs are detected as well.

As a final example, Fig. 3 shows the GC-MS traces obtained from the DMC and BF_3 -MeOH procedures applied to fatty acid analysis in coconut oil. The two chromatograms are qualitatively fully comparable, confirming the efficacy of the DMC/titanium silicate system for the generation of FAMES under Py-GC conditions.

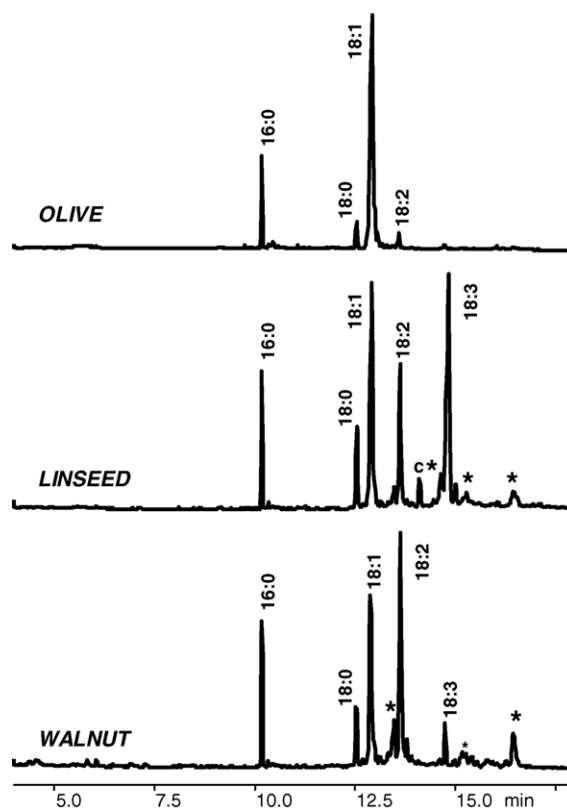


Fig. 2. Total ion chromatograms obtained from DMC/titanium silicate/Py-GC-MS of olive, linseed and walnut oil. (*) isomers of linoleic and linolenic methyl esters; c: contaminant.

3.2. Quantitative aspects

Data relative to the percentage distribution of FAMES in soybean oil determined by DMC/Py-GC-MS are presented in Table 1 along with the results deriving from the BF_3 -MeOH procedure and the values reported by the supplier. With respect to DMC/Py-GC-MS, the reference BF_3 -MeOH methodology provides data closer to the values reported by the supplier and with a better precision. The percentage abundance obtained from the alternative method reflects the expected composition in soybean oil, but with remarkable discrepancies. In particular, the relative content of polyunsaturated fatty acids (PUFAs), namely linoleic and linolenic acids, is biased towards lower values, whereas the abundance of saturated acids and oleic acid is concomitantly higher.

A similar behaviour is observed for other vegetable oils characterised by the same group of fatty acids, as evidenced

Table 1
Percentage distribution of FAMES obtained after Py-GC-MS of soybean oil in the presence of DMC and titanium silicate

FAME	DMC/Py, $n=6$	RSD (%)	BF_3 -MeOH, $n=3$	RSD (%)	Reported values
Palmitic	14 ± 1	7	12.1 ± 0.6	5	8.9
Stearic	7.2 ± 0.3	4	5.0 ± 0.3	6	3.8
Oleic	30 ± 2	7	23 ± 1	4	22.0
Linoleic	45 ± 2	4	54 ± 2	3	56.7
Linolenic	4.0 ± 0.4	10	5.5 ± 0.1	2	5.9

Comparison with the BF_3 -methanol procedure and values reported by the supplier.

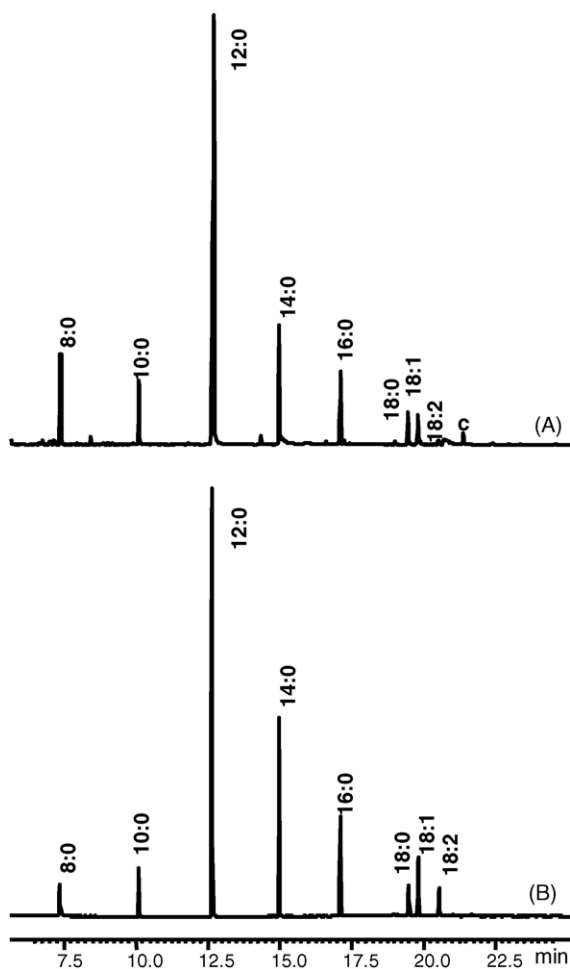


Fig. 3. Total ion chromatogram obtained from (A) DMC/titanium silicate/Py-GC-MS and (B) BF₃-methanol analysis of coconut oil; c: contaminant.

in Table 2. FAME patterns resulting from DMC/Py-GC-MS are skewed towards lower levels of PUFAs with respect to the BF₃-MeOH procedure. The systematic impoverishment of PUFAs is partly ascribed to the thermal isomerisation/chain migration of double bonds, as mentioned in Section 3.1.

However, Fig. 3 shows that when the oil is primarily composed of saturated fatty acids, as in the case of coconut plant, the FAME distribution is enriched in short chain components. This finding indicates that thermal degradation processes other than double bond isomerisation (e.g. mass discrimination) may affect the FAME distribution. Despite the weaknesses of DMC/Py-GC-MS in terms of accuracy, the FAME profiles

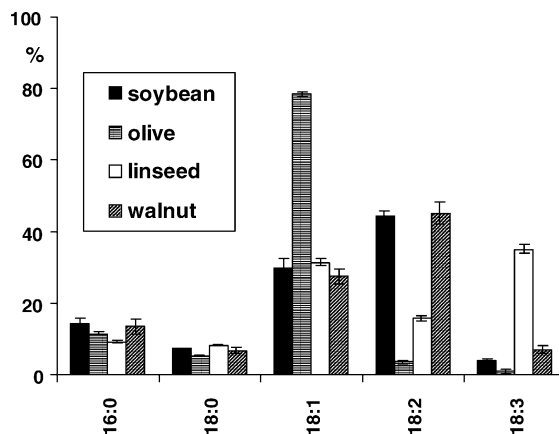


Fig. 4. Percentage FAME distribution resulting from DMC/titanium silicate/Py-GC-MS of soybean, olive, linseed and walnut oil.

produced by this technique are sufficiently representative to be adequate for identification purposes. This aspect is remarked in Fig. 4, showing the relative composition of the principal FAMES of four vegetable oils resulting from DMC/Py-GC-MS analysis. Each oil sample can be easily recognised by its own distribution of unsaturated fatty acids.

3.3. Procedural aspects

The DMC/Py and BF₃-MeOH procedures differ remarkably in the sample treatment required prior to GC analysis. The former is a one-step procedure, as the *trans*-methylation reaction takes place inside the pyrolyser unit and formed FAMES are directly swept into the GC-MS apparatus for separation and detection. As far as the nature of employed chemicals is concerned, the DMC/Py method is much less critical in terms of risk factors, as evidenced in Table 3. While DMC is classified as highly flammable, as methanol and *n*-hexane are, these latter solvents are in addition harmful and toxic. Furthermore, the smaller amount of employed chemicals and the reduced manual work-up in the DMC/Py method, may result in reduced waste generation and operative costs.

Concern may arise for the nanosized titanium silicate, as scientific knowledge on potential health adverse effects of nanoparticles is still limited [22]. Nonetheless, the particle size probably plays an important role in trapping DMC and/or promoting its methylating activity. In fact, the properties of ultra-fine particles can differ significantly from the coarser counterpart for

Table 2
Percentage distribution of FAMES obtained after Py-GC-MS of olive, linseed and walnut oils in the presence of DMC and titanium silicate

FAME	Olive		Linseed		Walnut	
	DMC/Py	BF ₃ -MeOH	DMC/Py	BF ₃ -MeOH	DMC/Py	BF ₃ -MeOH
Palmitic	11.6 ± 0.5	12.4 ± 0.1	9.3 ± 0.3	6.7 ± 0.2	13 ± 2	8.6 ± 0.4
Stearic	5.3 ± 0.2	3.8 ± 0.1	8.2 ± 0.2	4.6 ± 0.1	6.8 ± 0.9	2.9 ± 0.5
Oleic	78.5 ± 0.5	76 ± 1	31.5 ± 0.8	20.7 ± 0.6	27 ± 2	18 ± 1
Linoleic	3.6 ± 0.5	7.0 ± 0.9	15.8 ± 0.8	18.2 ± 0.7	45 ± 3	59 ± 2
Linolenic	1.0 ± 0.6	0.9 ± 0.2	35 ± 1	50 ± 2	7 ± 1	11.5 ± 0.5

Comparison with the BF₃-methanol procedure. Mean ± SD from three replicate analyses.

Table 3
Risk phrases and quantity of chemicals employed in DMC/Py–GC–MS and BF₃–MeOH methods

Method	Chemical	Approximate quantity	Risk phrases ^a
DMC/Py–GC–MS	Dimethyl carbonate	0.5 mL	R11
	Titanium silicate ^b	0.005 g	R36/37
BF ₃ –MeOH	NaOH	0.1 g	R35
	<i>n</i> -Hexane	>5 mL	R11; R38; R48/20; R51/53; R62; R65; R67
	BF ₃ –methanol ^b	5 mL	R20/22; R34; R48/23
	Methanol	>5 mL	R11; R23/24/25; R39/23/24/25

^a Information from European Chemical Substances Information System (<http://ecb.jrc.it/esis/>); R11: highly flammable. R34: causes burns. R38: irritating to the skin. R62: possible risk of impaired fertility. R:65 harmful: may cause lung damage if swallowed. R20/22: harmful by inhalation and if swallowed. R23/24/25: toxic by inhalation, in contact with skin and if swallowed. R36/37: irritating to eyes and respiratory system. R48/20: harmful: danger of serious damage to health by prolonged exposure through inhalation. R35: causes severe burns. R39/23/24/25: Toxic: danger of very serious irreversible effects through inhalation, in contact with skin and if swallowed. R48/23: toxic: danger of serious damage to health by prolonged exposure through inhalation. R51/53: toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment. R67: vapours may cause drowsiness and dizziness.

^b Not classified; risk statement from the supplier.

the same material [22]. Notably, the micro-porous Engelhard titansilicate 10 (ETS 10) was much less effective in promoting on-line pyrolytic *trans*-esterification of soybean oil than nanopowder titanium silicon oxide under the same conditions (data not shown). Besides particle size, the chemical nature of the catalyst is a crucial factor governing the *trans*-methylating efficiency of DMC, and the possible mechanism of FAME formation discussed in a previous publication for zeolite 13X probably holds plausible for titanium silicate as well [16]. The enhanced activity of titanium silicate over zeolite X might be also due to the stronger basicity of the former group of catalysts [23].

According to the proposed mechanism, the production of FAMEs occurs through several steps which include pyrolytical formation of fatty acid, acid–base reaction onto the solid surface of the catalyst, and nucleophilic attack of the carboxylate ion on the methyl group of activated DMC [16]. At this regard, it is worth pointing out that both thermal and chemical reactions are involved, thus the overall process could be more appropriately termed as thermochemolysis rather than pyrolysis.

4. Conclusions

The novel dimethyl carbonate/titanium silicate system was proved to be effective for the generation of FAMEs from triglycerides useful for one-step profiling of fatty acids in vegetable oils by means of a Py–GC apparatus. Although the proposed method did not compare favourably with well-established procedures in terms of precision and bias, nevertheless is attractive for the rapidity and ‘greenness’ of sample treatment, which simply requires the use of small quantity of the non-toxic/non-corrosive reagent DMC and nanopowder titanium silicate. The validity of the proposed method was tested on vegetable oils, which are soluble in DMC and did not contain potential interfering substances.

A more complete assessment of the proposed technique requires further investigations on the novel nanopowder titanium silicon oxide and similar nanostructured materials, in terms of catalytic behaviour, potential risks associated to nanoparticles, and potentiality for the analysis of more complex matrices.

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