Anal Bioanal Chem (2008) 392:505–514
DOI 10.1007/s00216-008-2274-7

ORIGINAL PAPER

Comparison of extraction and derivatization methods for fatty acid analysis in solid environmental matrixes

María Gómez-Brandón · Marta Lores · Jorge Domínguez

Received: 9 April 2008 / Revised: 24 June 2008 / Accepted: 1 July 2008 / Published online: 24 July 2008

Abstract This study involved comparison of different extraction and derivatization methods for determining FAs in soil and in four highly organic matrixes (cattle manure, pig slurry, compost, and vermicompost), by application of a multifactor categorical design. Although some studies have been carried out regarding the application of FA analysis to highly organic matrixes, comparison and verification are still required to test which methods of extraction and derivatization of FAs function best for these matrixes. We compared three extraction methods (one in which the same extraction mixture as used in the Folch method was employed, a modification of the Bligh and Dyer method, and a microwave-assisted extraction) and two derivatization procedures (alkaline methanolysis and derivatization with trimethylsulfonium hydroxide (TMSH)). The highest yields of FAs belonging to different structural classes, and of individual FAs used as microbial biomarkers were obtained by application of the same extraction mixture as in the Folch method and use of TMSH as derivatization agent. These methods also involved a significant reduction in the complexity and time involved in sample preparation.

Keywords Fatty acid extraction · Fatty acid derivatization · Soil · Compost · Vermicompost · Microbial biomarkers

M. Gómez-Brandón (✉) · J. Domínguez
Departamento de Ecología y Biología Animal,
Universidad de Vigo,
36310 Vigo, Spain
e-mail: mariagomez@uvigo.es

M. Lores
Departamento de Química Analítica, Nutrición y Bromatología,
Facultad de Química, Instituto de Investigación y Análisis
Alimentario (IIAA), Universidad de Santiago de Compostela,
Avda. das Ciencias s/n, Campus Sur,
15782 Santiago de Compostela, Spain

Introduction

The turnover of organic matter, nutrient cycling, and the fertility and quality of soils are mainly determined by the composition and activity of soil microbial communities, the diversity and functional significance of which are largely unknown [1]. Analysis of fatty acid (FA) composition has become an important tool for characterizing microbial communities, thereby eliminating the bias inherent in culture-based methods [2]. In addition, some FAs are biomarkers of specific microbial groups, which makes it possible to determine the presence and the abundance of these groups in their habitats [3].

Fatty acids are commonly extracted by use of mixtures of polar and non-polar organic solvents; this was first proposed by Folch et al. [4], who applied a chloroform-methanol mixture for the isolation of the total lipid content from animal tissues. Bligh and Dyer [5] employed a one-phase mixture containing chloroform, methanol, and water for total extraction of lipid from fish muscle. Modifications of this method have typically been used for extraction of FAs from environmental samples. The most widely used modifications include addition of a buffer (rather than water) to the extraction mixture. White et al. [6] added a phosphate buffer to study microbial communities in marine and estuarine sediments and Frostegård et al. [7] used a citrate buffer to study soil microbial communities. Another method of extraction based on the use of microwave irradiation has been used for isolation of lipids from biological tissues [8, 9] and fungal spores [10]. Recently, Lores et al. [11] employed this technique for extraction of FAs from environmental matrixes with high contents of organic matter.

Before analysis by gas chromatography-mass spectrometry (GC-MS), fatty acids are usually transformed into their

less polar methyl ester derivatives (FAMEs) by mild alkaline methanolysis, which is a base-catalyzed reaction [12, 13]. Hydroxides such as tetramethylammonium hydroxide (TMAH), trimethylsulfonium hydroxide (TMSH), and trimethylphenylamine hydroxide (TMPAH) have also been employed as derivatization agents for profiling FAs in bacteria [14], zooplankton [15], microalgae [16], polyunsaturated fatty acid rich oil [17], soil [18], and organic matrixes [11].

Although analysis of FAs in highly organic matrixes has already been carried out with the methods used for FA analysis in soil [19, 20], comparison and verification are still required to test which methods of extraction and derivatization of FAs function best for these matrixes. The aim of this study was to compare three extraction methods (one method using the same extraction mixture as in the Folch method [4]; the Bligh and Dyer method as modified by White et al. [6], and a microwave-assisted extraction) and two derivatization methods (alkaline methanolysis and derivatization with TMSH) for FA analysis in soil and four organic matrixes (cattle manure, pig slurry, compost, and vermicompost) by applying a multifactor categorical design.

Sample material

Chemicals

Standards of twenty characteristic FAMEs of the analyzed samples belonging to the 37-component FAME mix #47885-U (1000 μg mL^{-1}, Supelco, Bellefonte, USA) were used. The remaining standards (mixture BR1 #90–1051 (1000 μg mL^{-1}); methyl 13-methyltetradecanoate #21–1413 (250 μg mL^{-1}); methyl 15-methylhexadecanoate #21–1615 (250 μg mL^{-1}); methyl *cis*-9,10-methylenehexadecanoate #48–23–1709–7 (250 μg mL^{-1}); methyl *cis*-11,12-methyleneoctadecanoate #48–23–1911–7 (250 μg mL^{-1})) were supplied by Larodan Lipids (Malmö, Sweden). The internal standard methyl nonadecanoate (19:0, 230 μg mL^{-1}) and the derivatization agent TMSH (~0.25 mol L^{-1} in methanol) were purchased from Sigma-Aldrich (Madrid, Spain).

Chloroform, methanol, *n*-hexane and acetone were obtained from Scharlab (Barcelona, Spain), and methyl *tert*-butyl ether from Sigma-Aldrich. All reagents were HPLC-grade. Sodium sulfate (Na_2SO_4) and potassium hydroxide (KOH) were supplied by Scharlab (Barcelona, Spain), and the dibasic potassium phosphate (K_2HPO_4) used to prepare the phosphate buffer was purchased from Sigma-Aldrich. Milli-Q water was obtained by purifying demineralized water in a Milli-Q system (Millipore, Madrid, Spain).

Laboratory glassware was soaked overnight in phosphate-free Extran MA O3 5% (*v*/*v*) aqueous solution (Merck, Mollet del Valles, Barcelona, Spain), and thoroughly washed in tap and deionized water.

Real samples

Five different solid environmental matrixes were analyzed in the study: cattle manure and pig slurry, which were collected from farms in Zamanes and Tomiño (Galicia, NW Spain), respectively, soil collected from a field at the University of Vigo (Galicia, NW Spain), vermicompost produced in the laboratory by the earthworm *Eisenia andrei*, and compost derived from pruning waste, leaves and grass clippings. All samples were lyophilized before FA analysis.

Methods

Extraction of FAs from the different matrixes was carried out with three different methods, as summarized in Fig. 1.

Modified Folch method

Total FAs were extracted from 200 mg of each substrate with 60 mL chloroform-methanol, 2:1 (*v*/*v*), in 100-mL sterilized plastic jars. The jars were shaken vigorously for 30 min and the mixture was allowed to separate at room temperature for 24 h. The supernatant was filtered, collected in a glass test tube and then evaporated to dryness under a stream of oxygen-free N_2 gas. The same extraction mixture as described by Folch et al. [4] was used in this study, but without following the washing procedure; we therefore refer to the method as the modified Folch method.

Bligh and Dyer (B and D) method, as modified by White et al. [6]

In preliminary experiments we compared the solvent extraction mixtures chloroform-methanol-phosphate buffer, 1:2:0.8 (*v*/*v*/*v*) proposed by White et al. [6], and chloroform-methanol-citrate buffer, 1:2:0.8 (*v*/*v*/*v*) recommended by Frostegård et al. [7] for extraction of FAs from organic matrixes (cattle manure, pig slurry, compost, and vermicompost). We found no significant differences in the FA extraction yield, except for the fungal biomarker 18:2ω6c, for which with the phosphate buffer rendered higher yields (data not shown).

Total FAs were extracted from 200 mg of each substrate by addition of a single-phase mixture 1:2:0.8 (*v*/*v*/*v*) containing 1.9 mL chloroform, 3.75 mL methanol, and 1.5 mL phosphate buffer (50 mmol L^{-1}, pH 7.4). The suspension was mixed vigorously for 30 min, and then left

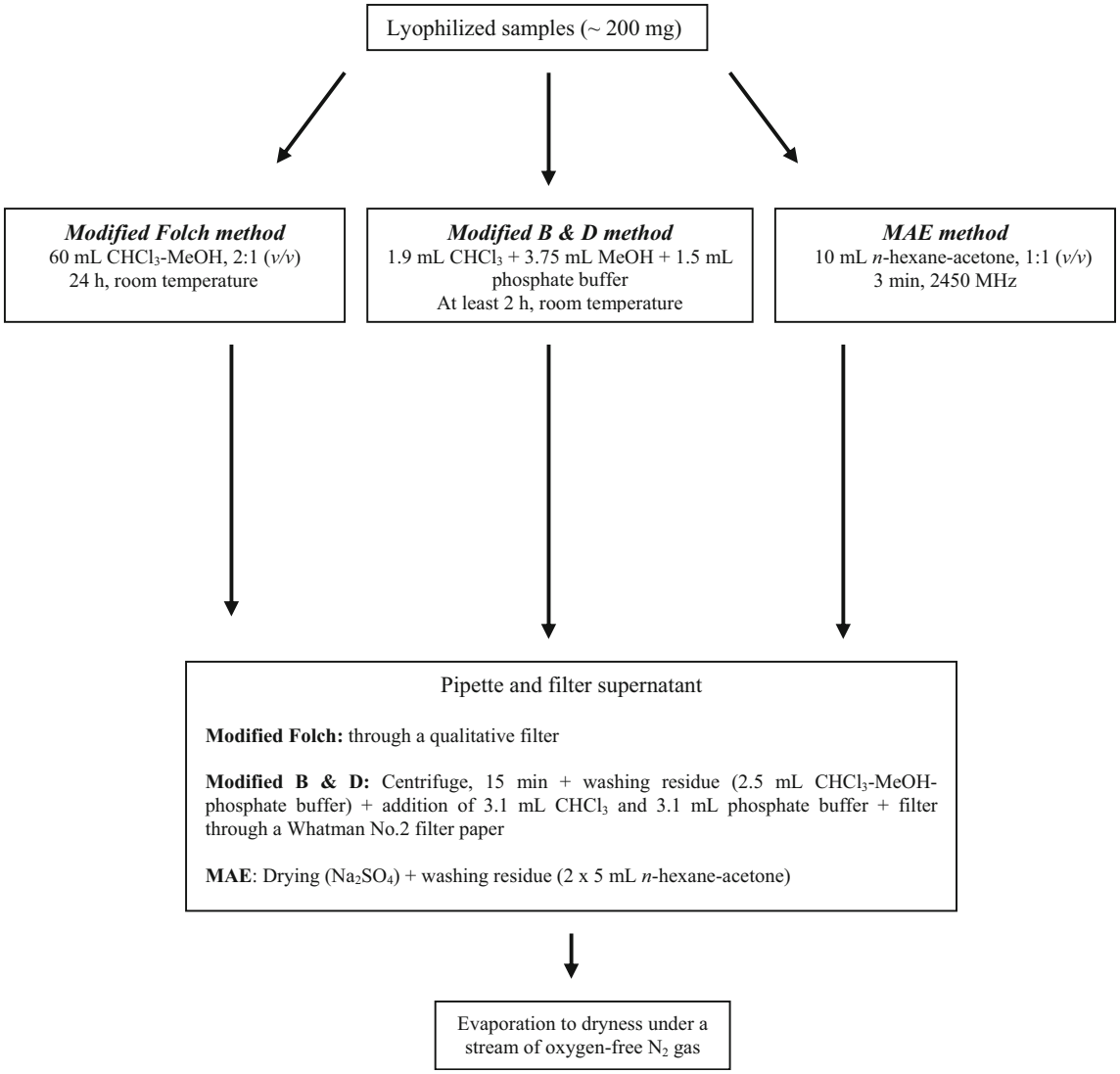

Fig. 1 Flow diagram of the three methods used for FA extraction in the different solid environmental matrixes

for at least 2 h to allow extraction of lipids. The samples were then centrifuged for 15 min at 2500 r.p.m. to separate the phases. The supernatant was collected in a glass test tube and the other phase was washed once with 2.5 mL extraction mixture. This suspension was then shaken and centrifuged as before, and the supernatant obtained was mixed with the first, before addition of 3.1 mL chloroform and 3.1 mL phosphate buffer. The suspension was again shaken and centrifuged. The further addition of chloroform improves the solubility of FAs whereas the phosphate buffer splits the phases into a lower phase containing the chloroform and an upper phase where possible non-lipid material dissolved in the previous steps is back-extracted from the chloroform layer to the upper phase. The upper aqueous layer was then removed and the chloroform phase was filtered. The chloroform layer was then transferred to a glass test tube and evaporated to dryness under a stream of oxygen-free N_2 gas.

Microwave assisted extraction (MAE)

In this study, total FAs were extracted from 200 mg of each substrate with 10 mL *n*-hexane-acetone, 1:1 (*v*/*v*) in Teflon vessels [21]. A solvent with a significant dielectric constant such as acetone and methanol must be used in MAE, when the matrix is relatively transparent. The problem with these pure solvents is that they can reach high temperatures, and consequently lead to degradation of the substrate; hence, it is common to add a transparent solvent. One of the most frequently used transparent solvents in MAE is hexane because few matrix-interfering compounds are extracted when it is added to the solvent extraction mixture. As

hexane and methanol are barely miscible, acetone is usually chosen in combination with hexane. These vessels were tightly sealed and placed in a scientific microwave oven (CEM Corporation MDS-2000) operating at 2450 MHz and 630 W maximum output, and irradiated at medium power (60% of maximum output power, manufacturer's setting) for 20 s three times, with 1 min cooling between each irradiation; these conditions were adjusted in order to obtain the most accurate response for this type of highly organic matrixes [10]. The samples were cooled for approximately 30 min and then allowed to settle. The supernatants from each vessel were separated, filtered through Na_2SO_4 (to dry them), and collected in a glass test tube. The residue from washing (2×5 mL *n*-hexane-acetone, 1:1 (*v*/*v*)) was also dehydrated through Na_2SO_4 [9]. The combined solutions were evaporated to dryness under a stream of oxygen-free N_2 gas.

FAMEs from the FA extracts, obtained with the three extraction methods described above, were prepared with two different derivatization procedures schematically presented in Fig. 2.

Alkaline methanolysis [13]

Total lipidic extracts were vortex mixed for 30 s in 2 mL of a mixture of methanolic KOH solution (0.2 mol L^{-1}) and toluene-methanol, 1:1 (*v*/*v*), incubated at 37 °C in a water bath for 15 min, cooled, and neutralized with acetic acid (1 mol L^{-1}). The FAMEs were then extracted as follows: hexane-chloroform, 4:1 (*v*/*v*), 2 mL, and Milli-Q water,

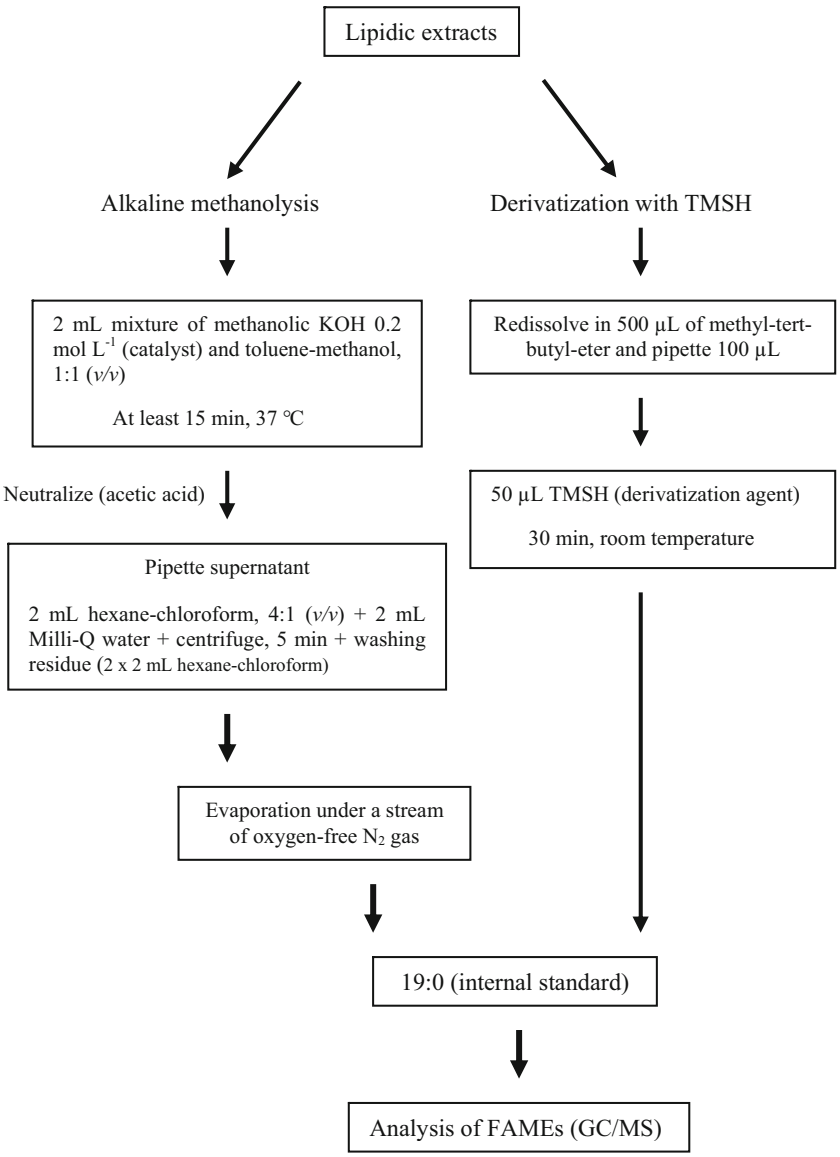

Fig. 2 Flow diagram of the two procedures used for FA derivatization in the different solid environmental matrixes

2 mL, were added to the samples, which were then centrifuged for 5 min at 2000 r.p.m. to separate the phases. The upper phase, which is the organic phase containing the FAMEs, was transferred to a glass test tube and the lower phase was washed twice with 2 mL hexane-chloroform, 4:1 (*v*/*v*). This sample was again shaken and centrifuged, and the supernatant obtained was mixed with the first, and then evaporated under a stream of oxygen-free N_2 gas to a volume of 0.5 mL. Prior to GC-MS analysis, 10 μL methyl nonadecanoate FA (19:0 at 230 μg mL^{-1}; Sigma-Aldrich) was added as an internal standard (IS) to 150 μL FAME extracts.

Derivatization with TMSH [9]

Total lipidic extracts were redissolved in 500 μL methyl *tert*-butyl ether. This solution (100 μL) was placed in a screw-cap vial with 50 μL derivatization agent (TMSH), vortex mixed for 30 s, and allowed to react for 30 min; 10 μL methyl nonadecanoate FA (19:0 at 230 μg mL^{-1}; Sigma-Aldrich) was then added as IS to 150 μL of the FAME extracts.

GC-MS conditions

GC-MS analysis of the FAME extracts was performed with a Varian 3800 gas chromatograph (Varian Chromatography Systems, Walnut Creek, CA, USA) equipped with a Saturn 2000 mass spectrometer (Varian Chromatography Systems). The system was operated by Saturn GC-MS Workstation v5.52 software. FAMEs were separated on a CP-SIL 88 Varian Select FAME FS 50 m × 0.25 mm × 0.2 μm capillary column (Varian Chromatography Systems). The GC oven temperature program was: 50 °C hold 2 min, rate 20° min^{-1} to 140 °C and then 3° min^{-1} to 250 °C. Helium (purity 99.999%) was used as the carrier gas, at a constant column flow rate of 1 mL min^{-1}. The injector was operated in splitless mode and programmed to return to the split mode 2 min after the beginning of a run. The split ratio was 1:50. The injector temperature was 280 °C. The mass spectrometer was operated in the electron ionization mode (70 eV). The mass range was scanned from 40 to 650 amu. Experimental conditions for ionization were: multiplier voltage, 1650 V; filament emission current, 10 μA; axial modulation voltage, 4 V; Trap, manifold and transfer line temperatures were 170, 70, and 280 °C, respectively. To identify the FAMEs, the retention times and the mass spectra were compared with those obtained from the standards. FAMEs were quantified by an internal standard calibration procedure, with 19:0 as IS. The calibration ranges of the FAMEs belonging to the 37-component FAME mix were 0.4–2 μg mL^{-1}, 0.5–4 μg mL^{-1}, and 1–6 μg mL^{-1} depending on the percentage by weight of each FAME within this mixture: 2, 4, and 6% respectively. The calibration curves for FAMEs belonging to the BR1 mixture were prepared in the range 1–35 μg mL^{-1}; and the calibration ranges of the remaining standard FAMEs were set between 50 and 250 μg mL^{-1}. The coefficients of determination (R^2) were higher than 0.99 for all calibration curves. FAMEs were described by the standard ω-nomenclature A:BωC [22].

Experimental design

The studied factors were type of matrix (A), with five levels (soil, cattle manure, pig slurry, compost, and vermicompost); extraction method (B), with three levels (one modification of the Folch method; one modification of the B and D method and MAE); and derivatization method (C), with two levels (alkaline methanolysis and derivatization with TMSH). A multifactor categorical design {5×3×2}, with 30 runs, was selected. This type of design consists of all levels of combinations of two or more non-quantitative factors, where the user sets the number of levels.

The response variables analyzed were the total amount of FAs (total FAs) expressed as the sum of the concentrations (μg mL^{-1}) of all identified FAs; and five other dependent variables calculated as the sum of the content (μg mL^{-1}) of the FAs belonging to the same structural class, i.e., straight-chain saturated FAs (ΣSFAs); straight-chain monounsaturated FAs (ΣMUFAs); straight-chain polyunsaturated FAs (ΣPUFAs); *iso*/*anteiso* branched-chain saturated FAs (Σ*iso*/*anteiso* FAs); and cyclopropyl branched-chain saturated FAs (Σcyclopropyl FAs). The *iso*/*anteiso* FAs are considered biomarkers for Gram-positive bacteria, and cyclopropyl FAs used as biomarkers for Gram-negative bacteria [3, 23]. Because 18:2ω6c is considered to be mainly of fungal origin [24] we also included this PUFA as an additional variable in the analysis.

The results obtained were evaluated by analysis of variance (ANOVA), which measures whether a factor contributes significantly to the variance of the response. The second-order interactions between the main factors were also determined to avoid coming to mistaken conclusions when the effect of a factor can be altered by other factors. A comparison of means based on Tukey's test was used to determine significant differences between the levels within each factor. The experimental design and the data analysis were performed using Statgraphics Plus v5.1 software [25].

Results and discussion

Twenty-nine saturated and unsaturated FAs, ranging from 10 to 20 carbon atoms were identified in this study from their corresponding retention times and identification ions;

they were also quantified using the selected quantification ions (Table 1). All FAs were obtained in the same chromatographic run applying the optimized method detailed in the Experimental section. The power and sensitivity of this method to analyze the studied FAs is clearly shown in the ion chromatograms of Fig. 3, showing that the peaks of FAs belonging to the different structural classes were adequately resolved under the applied chromatographic conditions. The standard mixture 37-component FAME mix was used to build the ion chromatograms of SFAs, MUFAs, and PUFAs (Fig. 3a-c). For the ion chromatogram of branched-chain saturated FAs (Fig. 3d), the chromatograms of different standard FAMEs (see Experimental section) were overlaid: mixture BR1, i15:0, i17:0, cy17:0, and cy19:0.

Table 1 Retention time (t_R, min), molecular weight (MW), and identification and quantification ions (m/z) of the twenty-nine FAs included in the analysis

FAMEs	t_R (min)	MW	Identification and quantification ions (m/z)
Straight-chain saturated			
10:0	14.522	186	74/87/143
11:0	15.888	200	55/74/87
12:0	17.427	214	55/74/87
13:0	19.130	228	74/87/143
14:0	20.966	242	74/87/143
15:0	22.914	256	55/74/87/143
16:0	24.906	270	55/74/87/143
17:0	26.963	284	55/74/87/143
18:0	28.998	298	74/87/143/298
19:0 (IS)	30.926	312	312
20:0	32.998	326	74/87/143/326
Straight-chain monounsaturated			
14:1ω5c	22.014	240	55/67/69
15:1ω5c	24.080	254	55/67/69/96
16:1ω7c	25.832	268	55/67/69
17:1ω7c	27.909	282	55/67/69
18:1ω9t	29.479	296	55/67/69/83
18:1ω9c	29.783	296	55/67/69/83
Straight-chain polyunsaturated			
18:2ω6t	30.399	294	67/81/95
18:2ω6c	31.116	294	67/81/95
18:3ω6c	31.998	292	79/67/91/93
18:3ω3c	32.694	292	79/67/91/93
Branched-chain saturated			
iso/*anteiso*			
i14:0	20.073	242	74/87/199
i15:0[a]	22.145	256	55/74/143/213/257
a15:0	22.237	256	55/74/87/199
i16:0	23.942	270	55/74/87/227
i17:0[a]	26.164	284	55/74/143/241/285
a17:0	26.375	284	55/74/87/143/284
Cyclopropyl			
cy17:0	27.790	282	55/69/81/97/250
cy19:0	31.817	310	55/69/83/97/278

[a] The pseudomolecular ion $[M + 1]^+$ was selected as one of the identification and quantification ions because of its high relative abundance under the experimental conditions used

Fatty acid yields differed greatly depending on the type of matrix, and both the extraction and derivatization method had different effects on the total amount of FAs; the yields of all the structural classes of FAs (with the exception of cyclopropyl FAs); and the concentration of the fungal biomarker 18:2ω6c (Table 2). Besides determining the influence of each factor on FA yields, this design also permitted us to evaluate the two-factor interactions (Table 2). There was no significant interaction between the type of matrix and the extraction method; however, the effect of the derivatization method depended on the type of matrix and on the extraction method for total FAs, ΣSFAs and Σ*iso*/*anteiso* FAs (Table 2). A significant interaction between the extraction method and the derivatization procedure was also found for ΣPUFAs and the fungal biomarker 18:2ω6c (Table 2). Therefore, it is evident that the choice of the extraction and the derivatization method plays an important role in determining the total amount of FAs, and when FAs are also analyzed by structural classes or used are biomarkers in the studied matrixes.

The total amount of FAs and the extraction yields of the different structural classes of FAs and of the fungal biomarker 18:2ω6c were much higher with the modified Folch method than with the other two extraction methods, irrespective of the type of matrix (Fig. 4). The modified Folch method rendered higher yields of FAs than the B and D method as modified by White et al. [6]; the main difference between these methods is the absence or presence of a buffer in their extraction mixtures. A buffered solvent system is usually used in samples containing different amounts of salts (i.e., sediments with a high mineral content) in order to prevent ionic adsorption effects [26]; White et al. [6] included a phosphate buffer in the solvent mixture for analysis of FAs in marine and estuarine sediments. However, in this study addition of a buffer did not improve the extraction yield of FAs, mainly because four of the matrixes studied are highly organic, and as such the buffer effect was not significant; the same occurred with the soil, probably because it is rich in organic matter. The longer incubation time (24 h) involved in the modified Folch method and the larger volume of extraction solvent mixture used may also have contributed, because larger yields of FAs were obtained with this method. Moreover, the reduced manual labor required may result in lower operating costs.

The yields of FAs extracted with MAE were also much lower than those obtained by the modified Folch method. The results may be attributed to the type of extraction

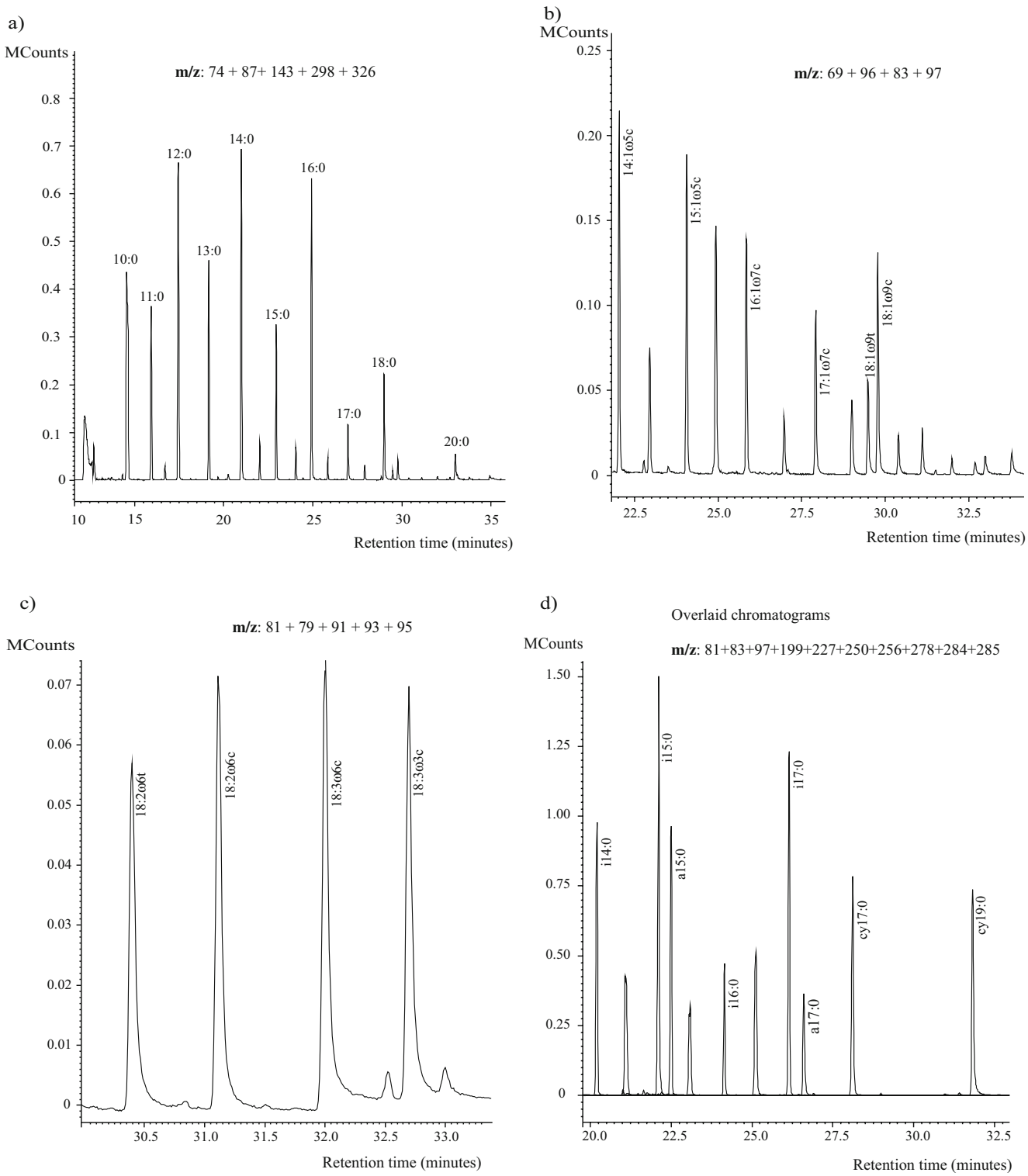

Fig. 3 Selected ion chromatograms for the (**a**) straight-chain saturated; (**b**) monounsaturated; (**c**) polyunsaturated; and (**d**) branched-chain saturated FAs

solvent mixture (chloroform-methanol or *n*-hexane-acetone), irrespective of the extraction procedure. A solvent mixture that is sufficiently polar to extract the FAs derived from cell membrane lipids (i.e. phospholipids), and sufficiently non-polar to obtain the FAs from neutral lipids, is required to achieve efficient extraction of FAs from environmental samples. According to Kates [27], inclusion of an alcohol in the extraction mixture is crucial for dissolution of the

Table 2 Main effects of the type of matrix and both the extraction and derivatization methods on the different structural classes of FAs and specific biomarker FAs. The second order interactions between the main factors are also shown

Dependent variables[a]		Main effects			Interactions		
		Matrix (A)	Extraction method (B)	Derivatization method (C)	AB	AC	BC
Total FAs	F-ratio	10.27	22.81	34.47	1.94	5.44	7.30
	P-value	**0.003**	**0.0005**	**0.0004**	0.185	**0.021**	**0.016**
ΣSFAs	F-ratio	7.72	59.16	68.70	0.89	7.16	10.88
	P-value	**0.008**	**0.0001**	**0.0001**	0.563	**0.009**	**0.005**
ΣMUFAs	F-ratio	6.96	8.44	24.64	1.53	3.46	3.48
	P-value	**0.010**	**0.011**	**0.001**	0.279	0.064	0.082
ΣPUFAs	F-ratio	3.98	10.17	13.83	1.50	2.65	6.82
	P-value	**0.046**	**0.006**	**0.006**	0.291	0.112	**0.019**
Σiso/anteiso FAs	F-ratio	7.57	10.72	15.18	2.31	3.96	4.60
	P-value	**0.008**	**0.006**	**0.005**	0.129	**0.047**	**0.047**
Σcyclopropyl FAs	F-ratio	24.73	0.63	4.81	2.35	4.31	0.73
	P-value	**0.0001**	0.555	0.060	0.124	0.303	0.512
Fungal biomarker 18:2ω6c	F-ratio	4.48	10.19	15.12	1.50	3.15	6.89
	P-value	**0.034**	**0.006**	**0.005**	0.291	0.079	**0.018**

Numbers in bold denote a significant effect at 95% confidence level

[a] Dependent variables, where Total FAs: the sum of all identified FAs; ΣSFAs: the sum of straight-chain saturated FAs; ΣMUFAs: the sum of straight-chain monounsaturated FAs; ΣPUFAs: the sum of straight-chain polyunsaturated FAs; *Σiso/anteiso* FAs: the sum of iso/anteiso branched-chain saturated FAs; Σcyclopropyl FAs: the sum of cyclopropyl branched-chain saturated FAs

The FA 18:2ω6c was analyzed within ΣPUFAs and also as an additional variable because of its great importance as a fungal biomarker

polar lipids in cell membranes, thereby facilitating extraction of FAs from these lipids.

When TMSH was used as the derivatization agent, the conversion of all FAs into FAMEs was much greater, especially in pig slurry, cattle manure, and vermicompost, than obtained by alkaline methanolysis (Fig. 5a), and whereas alkaline methanolysis converted similar amounts of FAs to FAMEs in each different matrix, derivatization with TMSH not only rendered different yields, depending on the source of the FAs, but also significantly higher concentrations in all cases (Fig. 5b). In addition, the combination of extraction with the modified Folch method and derivatization with TMSH was the best procedure for analyzing the different structural classes of FAs (Fig. 6a), including specific microbial biomarkers (Fig. 6b) in the solid environmental matrixes under study.

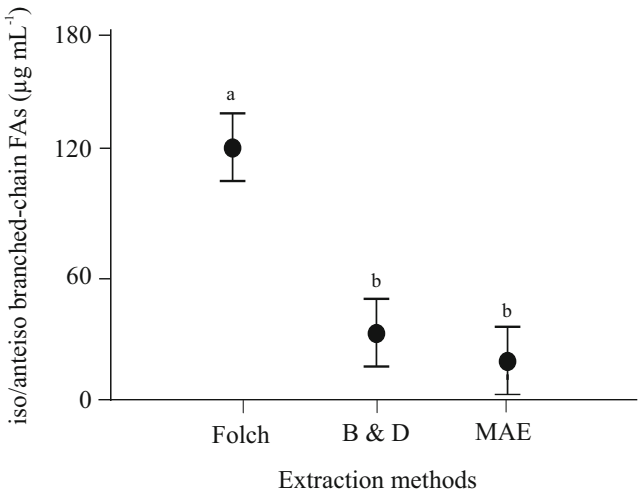

Fig. 4 Values (mean ± standard error) of the extraction yield of *iso/anteiso* branched-chain saturated FAs (μg mL^{-1}) obtained with the modified Folch and Bligh and Dyer methods and microwave-assisted extraction. *Different letters* above the error bars indicate significant differences at $P<0.05$ (Tukey's HSD test)

The great advantages of TMSH derivatization are that it is a simple and a non-time-consuming procedure. Esterification with TMSH can be performed at room temperature in a fast, single-step reaction, which consists of the formation of trimethylsulfonium salts by deprotonation of FAs. These salts are then thermally decomposed by heating, giving FAMEs and dimethyl sulfide ($(CH_3)_2S$). The by-products of this reaction (water and dimethyl sulfide) elute with the solvent peak (during the delay period) and do not disturb chromatographic separation of the analytes [28].

Despite its high alkalinity ($pK_b=12$), TMSH has been reported to be the best choice for methylation of PUFAs in comparison with other derivatization reagents such as TMAH and TMPAH. This is because trimethylsulfonium salts decompose at lower temperatures (approximately 200 °C), which thus lessens the degradation/isomerization side-reactions of PUFAs [29]. This could explain why the yields of PUFAs (and also the remaining FAs) were

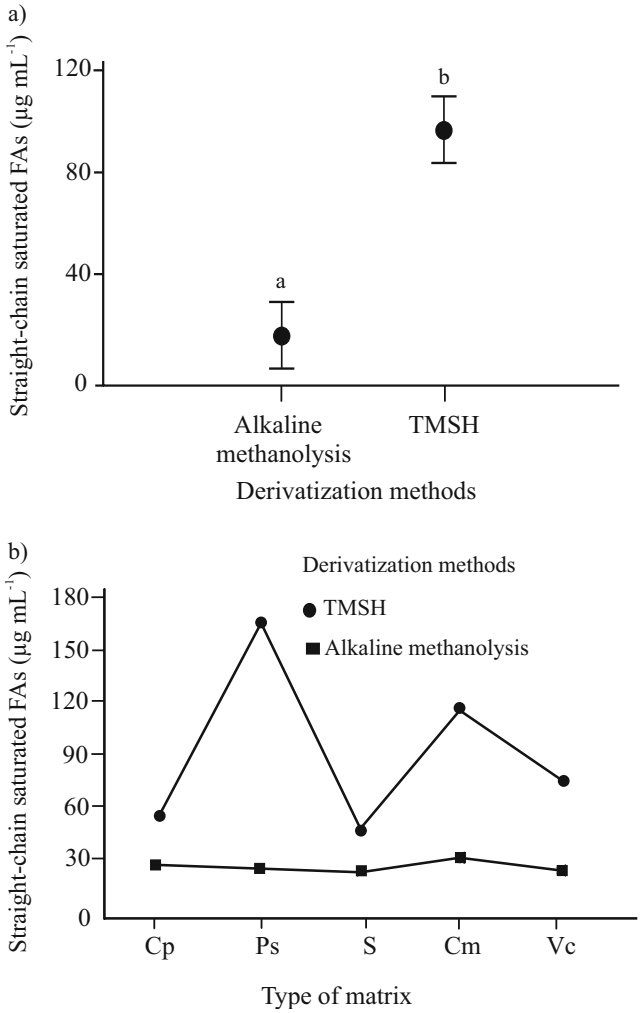

Fig. 5 (**a**) Values (mean ± standard error) of the yield of straight-chain saturated FAs (μg mL^{-1}) after derivatization with alkaline methanolysis and TMSH. *Different letters* above the error bars indicate significant differences at $P<0.05$ (Tukey's HSD test). (**b**) Matrix-derivatization interaction plot for straight-chain saturated FAs. The matrixes analyzed were cattle manure (*Cm*), pig slurry (*Ps*), soil (*S*), compost (*Cp*), and vermicompost (*Vc*)

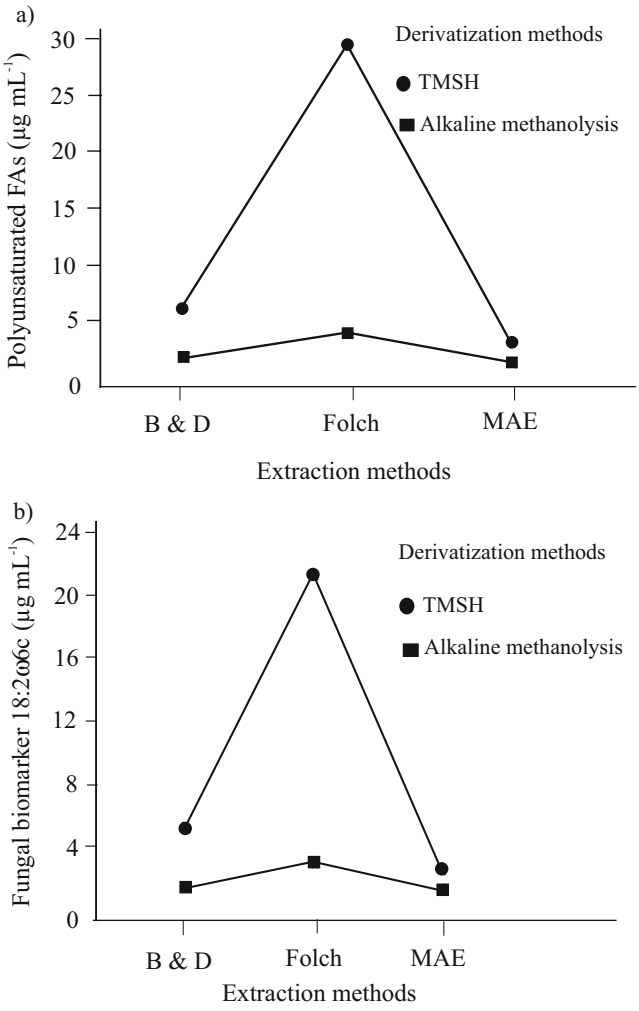

Fig. 6 Extraction-derivatization interaction plots for (**a**) straight-chain polyunsaturated FAs, and (**b**) the fungal biomarker 18:2ω6c

significantly higher when TMSH was used as the derivatization agent in this study (Fig. 5). Moreover, TMSH is stable in methanol for about six months when stored below 10 °C [28]; however, the methanolic potassium hydroxide (KOH) solution must be prepared immediately before use because KOH is hygroscopic.

The best-characterized reagent known to result in efficient methyl esterification of cyclopropyl FAs is sodium methoxide in anhydrous methanol. Sodium (or potassium) hydroxide in methanol used as catalyst in alkaline methanolysis is also suitable for this purpose [30]. However, in the present study, use of TMSH as the derivatization reagent did not result in significant differences in the yields of cyclopropyl FAs with respect to alkaline methanolysis.

Conclusion

This comparative study enabled us to test the efficiency of different methods for extraction and derivatization of FAs in five solid environmental matrixes. This was extremely useful for establishing the optimum method for analyzing FA profiles in these types of matrix. The modified Folch method rendered, irrespective of the type of matrix, the largest total amount of FAs and the highest yields of the different structural classes of FAs and the fungal biomarker 18:2ω6c. The largest conversion yields of all FAs into FAMEs were achieved using TMSH as derivatization agent; although its effect was greater in pig slurry, cattle manure, and vermicompost. The modified Folch method together with derivatization with TMSH was found to be the best option, rendering the highest yields of FAs. Moreover this combination of methods significantly reduced the complexity and time of sample preparation.

Acknowledgements This research was financially supported by the *Xunta de Galicia* (Project 07MRU023383PR). María Gómez Brandón is financially supported by an FPU fellowship from the Spanish Ministerio de Educación. We thank Salome Álvarez for helping with laboratory work. The authors whish to thank the two anonymous reviewers for helping us to improve the quality of this work.

References

1. Hill GT, Mitkowski NA, Aldrich-Wolfe L, Emele LR, Jurkonie DD, Ficke A, Maldonado-Ramirez S, Lynch ST, Nelson EB (2000) Appl Soil Ecol 15:25–36
2. Tunlid A, White DC (1992) Soil Biochem 7:229–262
3. Zelles L (1999) Biol Fert Soils 29:111–129
4. Folch J, Lees M, Stanley GHS (1957) J Biol Chem 226:497–509
5. Bligh EG, Dyer WJ (1959) Can J Biochem Phys 37:911–917
6. White DC, Davis WM, Nickels JS, King JD, Bobbie RJ (1979) Oecología 40:51–62
7. Frostegård Å, Tunlid A, Bååth E (1991) J Microbiol Methods 14:151–163
8. Vetter W, Weichbrodt M, Hummert K, Glotz D, Luckas B (1998) Chemosphere 37:2439–2449
9. Batista A, Vetter W, Luckas B (2001) Eur Food Res Technol 212:377–384
10. Young JC (1995) J Agric Food Chem 43:2904–2910
11. Lores M, Gómez-Brandón M, Pérez-Díaz D, Domínguez J (2006) Soil Biol Biochem 38:2993–2996
12. Dowling NJE, Widdel F, White DC (1986) J Gen Microbiol 132:1815–1825
13. White DC, Ringelberg DB (1998) In: Burlage RS, Atlas R, Stahl D, Geesey G, Sayler G (eds) Techniques in microbial ecology. Oxford University Press, New York, pp 255–272
14. Müller KD, Schmid EN, Kroppenstedt RM (1998) J Clin Microbiol 36:2477–2480
15. Nakanishi O, Yasuyuki I, Hirao S, Tsuge S, Ohtani H, Urabe J, Sekino T, Nakanishi M, Kimoto T (2003) J Anal Appl Pyrol 68:187–195
16. Blokker P, Pel R, Akoto L, Brinkman U, Vreuls R (2002) J Chromatogr A 959:191–201
17. Jun-Kai D, Wei J, Tian-Zhi Z, Ming S, Xiao-Guang Y, Chui-Chang F (1997) J Anal Appl Pyrol 42:1–8
18. Jandl G, Leinweber P, Schulten H-R, Ekschmitt K (2005) Soil Biol Biochem 37:1033–1041
19. Ranneklev SB, Bååth E (2003) Appl Environ Microb 69:3532–3539
20. Kato K, Miura N, Tabuchi H, Nioh I (2005) Biol Fert Soils 41:399–410
21. López-Ávila V, Young R, Beckert WF (1994) Anal Chem 66:1097–1106
22. IUPAC-IUC (1977) Eur J Biochem 79:11–21
23. O'Leary WM, Wilkinson SG (1988) In: Ratledge C, Wilkinson SG (eds) Microbial lipids. Academic Press, London, pp 117–202
24. Frostegård Å, Bååth E (1996) Biol Fert Soils 22:59–65
25. Statgraphics-Plus (2003) Experimental design, appendix C. Manugistics Inc, Rockville MD
26. Christie WW (1993) In: Christie WW (eds) Advances in lipid methodology-two. The Oily Press Ltd, Dundee, pp 95–213
27. Kates M (1986) Techniques of lipidology: isolation, analysis and identification of lipids. Elsevier, Amsterdam
28. Yamauchi K, Tanabe T, Kinoshita M (1979) J Org Chem 44:638–639
29. Estévez SL, Helleur R (2005) J Anal Appl Pyrol 74:3–10
30. Grogan D, Cronan J (1997) Microbiol Mol Biol R 61:429–441