Trans-Esterification of Fatty Acids from Microorganisms and Human Blood Serum by Trimethylsulfonium Hydroxide (TMSH) for GC Analysis

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Key Words

Gas chromatography
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FAME analysis
Bacteria
Blood serum

Summary

Trimethylsulfonium hydroxide (TMSH) can convert fatty acids into the corresponding fatty acid methyl esters (FAMEs) in a single step. These fatty acids may also be bound in biomolecules such as phospholipids and/or glycerides. Complex mixtures of saturated and unsaturated FAMEs which may contain hydroxy and cylopropyl groups are obtained by trans-esterification; they can easily be separated in most cases by capillary GC. When FAMEs are generated from different microorganisms e.g. bacteria the patterns of the chromatograms are characteristic. Examples of characteristic patterns of bacteria with different cell wall structures are shown. The described method of trans-esterification can also be applied directly to blood serum without sophisticated sample pretreatment. The profiles of the chromatograms match well those described in the literature obtained by other methods of trans-esterification or sample preparation.

Introduction

Capillary gas chromatography is the method of choice for the separation of complex isomeric mixtures in general because of the high efficiency which can be achieved in such columns with stationary phases of conventional polarity. The FAMEs are liberated from clinical materials like blood serum and isolates of microorganisms have a composition which is characteristic for such materials [1]. The variety of the methyl esters of different cell-bound fatty acids found, for example, in microorganisms produces profiles of the chromatograms which can be used for fingerprint identification of these organisms. This well known method can be used in microbiology for taxonomic characterization but has not succeeded in clinical microbiology up to now because of the time-consuming and laborious sample preparation which may take as long as an hour [2].

TMSH is suited to form FAMEs immediately from lipids at room temperature and has been successfully applied for sample preparation in the analysis of fats and oils in food chemistry [3–5]. It was to be expected that TMSH would also be suitable for applications in microbiology and clinical chemistry.

Experimental

Preparation of Sample Material

Enterococcus faecalis was grown on Trypticase soy broth agar (TSBA) [2], *Bacteroides thetaiotaomicron* on TSBA, supplemented with cysteine chloride as described [6]. Blood sera were obtained from healthy blood donors.

Lysis of Bacteria and Trans-Esterification of Fatty Acids

Lysis of bacteria and trans-esterification was carried out according to Müller et al. [6]. The whole procedure was performed in a GC vial. Only a few colonies of bacteria were transferred by a 1 µL loop from an agar plate and suspended in 10 µL distilled water. Other methods require the conversion of larger amounts of bacteria [2]. By adding 30 µL methanolic TMSH solution (0.2 M) bacteria are immediately lysed, and the cell-bound fatty acids converted into FAMEs. Blood serum (50 µL) was directly treated with 30 µL TMSH solution. In each case the reaction mixture was dried in a nitrogen stream, the remainder containing the FAMEs was diluted to 200 µL with a *tert*-butyl-methyl ether/methanol mix (10 : 1, v/v). The solution obtained was directly introduced into a standard capillary GC system using split injection.

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Instrumentation

FAME analyses were executed on a HP 5890 gas chromatograph equipped with split injector and FID (flame ionization detector). Bacterial FAMEs were separated on a 50 m fused silica column, SE 54 (crosslinked), id 0.25 mm, df 0.25 µm, carrier gas: 1.2 bar hydrogen, sample volume: 0.3 µL, split 1 : 30. Temperature: 150–270 °C, 4 °C min^{-1}. Serum FAMEs were separated on a 50 m fused silica column, OV-1 (crosslinked), id 0.18 mm, df 0.21 µm, carrier gas: 2.5 bar hydrogen, sample volume: 0.3 µL, split: 1 : 30. Temperature: 150–260 °C, 4 °C min^{-1} and 260–300 °C, 10 °C min^{-1}; 15 min isothermal. Both set of parameters proved to be suited to bacterial as well as serum FAMEs.

Results and Discussion

Identification of bacteria by FAME analysis is a well established method of medical microbiology [7]. Obviously, the broad application of this technique up to now has been prevented by the laborious and time-consuming sample preparation. In a number of steps bacteria are usually lysed, saponified, methylated and ether/hexane extracted at different temperatures. These procedures take about one hour [2]. In contrast, trans-esterification with TMSH is a one step method which allows the reproducible preparation of bacterial FAMEs within two minutes at room temperature. TMSH effects an alkaline lysis of cells. An excess of the reagent does not disturb the subsequent analysis. It is converted in the injector into volatile byproducts which are removed from the GC-system. Trans-esterification with TMSH can be performed in the micro-scale with very small amounts of sample.

The FAME profiles of *Bacteroides thetaiotaomicron* (Figure 1) and *Enterococcus faecalis* (Figure 2) were chosen to demonstrate the usefulness of the method for its application to gram-negative and gram-positive bacteria, representing types of bacteria with different cell wall structures.

All the different cellular fatty acids, which are significant for the identification of bacteria, can be separated and identified. In *Bacteroides thetaiotaomicron* (Figure 1) straight-chain, branched, saturated, unsaturated and hydroxy fatty acids could be resolved and identified, for example. The sample preparation method proposed here is also suited to the undisturbed isolation and determination of cyclopropyl (Figure 2) and hydroxy fatty acids. The latter are difficult to analyse with conventional methods as shown by other authors [8]. The method is very reproducible with regard to the relative peak areas in the chromatograms, i.e., the quantitative composition of FAMEs. The FAME profiles obtained by our sample preparation method do not appreciably differ from those obtained by conventional methods of sample preparation [6].

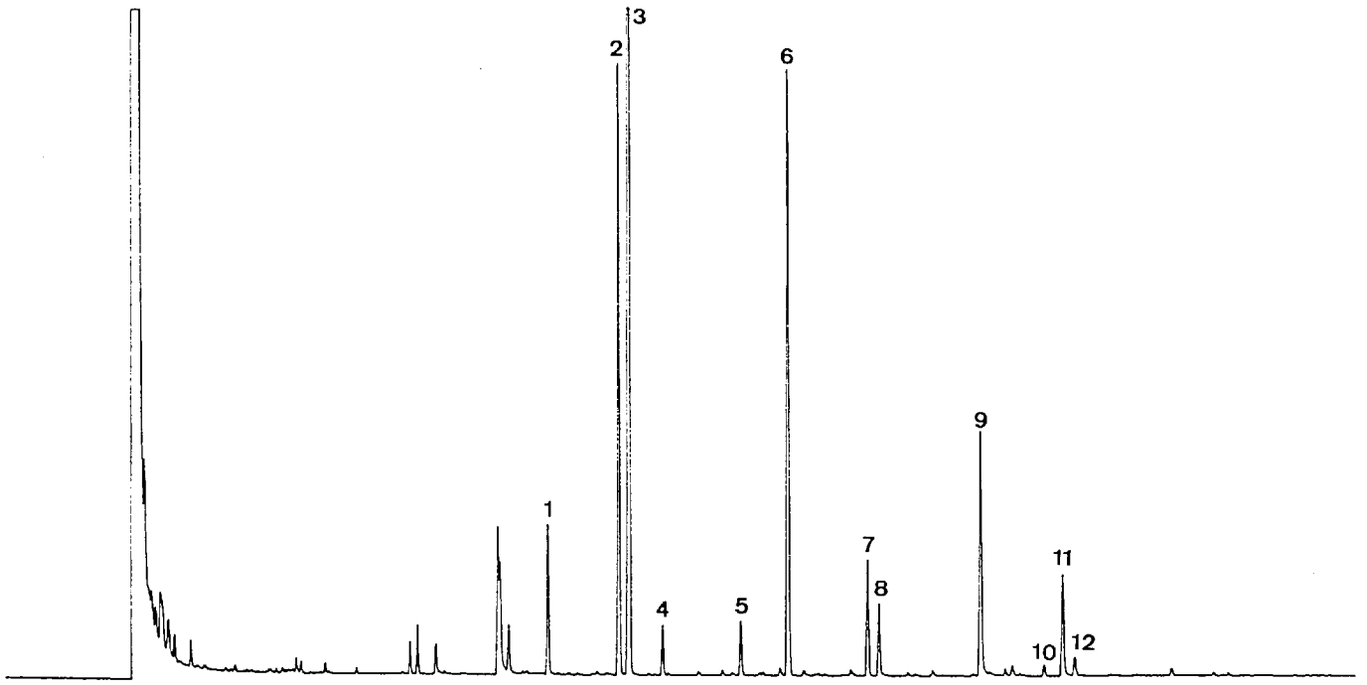

Figure 1

FAME Profile of *Bacteroides thetaiotaomicron*

Sample: 0.3 µl solution of FAMEs obtained by trans-esterification with TMSH (trimethylsulfonium hydroxide), split ratio 1 : 30.
1 C14 : 0; **2** i-C15 : 0; **3** a-C15 : 0; **4** C15 : 0; **5** i-C16 : 0; **6** C16 : 0; **7** i-C17 : 0; **8** a-C17 : 0; **9** C16 : 0, 3-OH; **10** C18 : 0; **11** i-C17 : 0, 3-OH; **12** a-C17 : 0, 3-OH

Column: 50 m methylphenyl polysiloxane SE 54 on FS, 0.25 mm i.d., film thickness 0.25 µm

Temperature: 150–270 °C, 4 °C min^{-1}

Carrier gas: 0.12 MPa H_2 Analysis time: 30 min

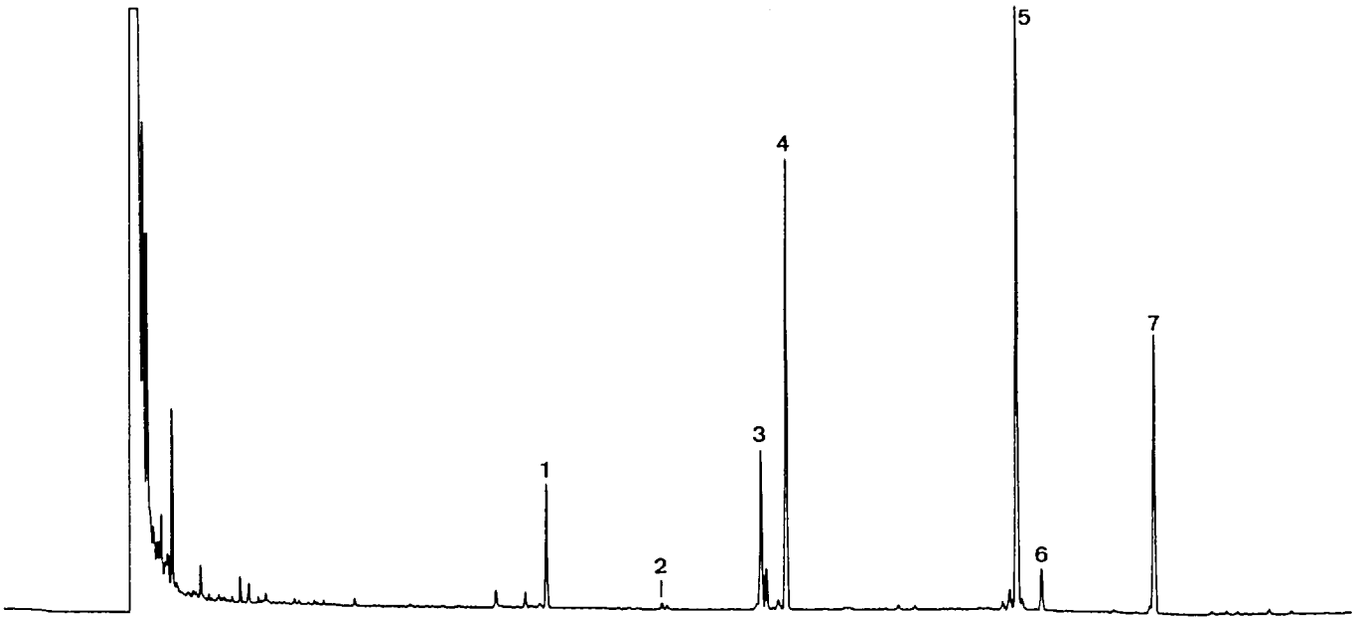

Figure 2
FAME Profile of *Enterococcus faecalis*
Sample: 0.3 µl solution of FAMEs obtained by trans-esterification with TMSH (trimethylsulfonium hydroxide), split ratio 1 : 30.
1 C14 : 0; **2** C15 : 0; **3** C16 : 1, cis 9; **4** C16 : 0; **5** C18 : 1, cis 9; **6** C18 : 0; **7** C19 : 0, cyclopropane
Column: 50 m methylphenyl polysiloxane SE 54 on FS, 0.25 mm i.d., film thickness 0.25 µm
Temperature: 150–270 °C, 4 °C min^{-1}
Carrier gas: 0.12 MPa H_2 Analysis time: 30 min

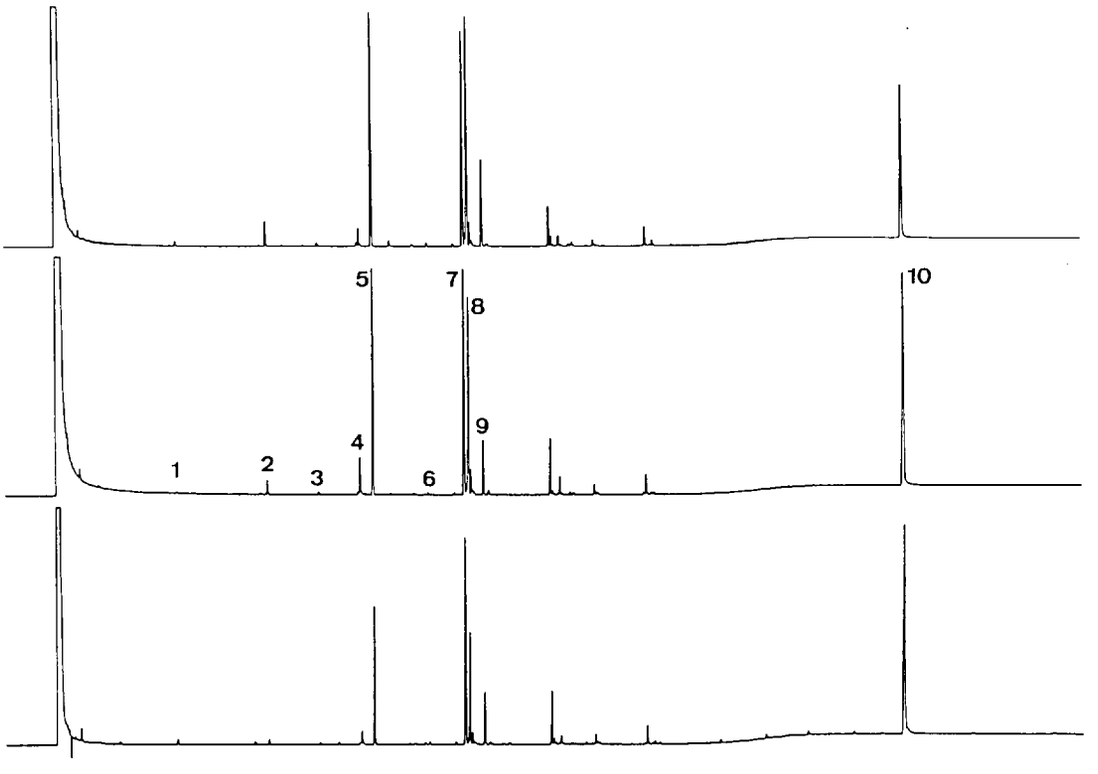

Figure 3
FAME Profiles of Human Blood Sera originating from different Blood donors
Sample: 0.3 µl solution of FAMEs obtained by trans-esterification with TMSH (trimethylsulfonium hydroxide), split ratio 1 : 30.
1 C12 : 0; **2** C14 : 0; **3** C15 : 0; **4** C16 : 1; **5** C16 : 0; **6** C17 : 0; **7** C18 : 2; **8** C18 : 1; **9** C18 : 0; **10** cholesterol
Column: 50 m methylpolysiloxane OV 1, crosslinked, on FS, 0.18 mm i.d., film thickness 0.21 µm
Temperature: 150–260 °C, 4 °C min^{-1}; 260–300 °C, 10 °C min^{-1}; 15 min isothermal
Carrier gas: 0.25 MPa H_2 Analysis time: 50 min

On account of their chemical lability (catalytic and thermal) samples containing hydroxy fatty acids must generally be injected under adequately different conditions in order to reveal the formation of artificial peaks. This problem of formation of secondary compounds from 3-OH fatty acids is under investigation currently.

A useful further application of the described TSMH procedure is the rapid analysis of FAMEs in blood serum and other clinical specimens such as liquor cerebrospinalis. Figure 3 shows typical fatty acid profiles obtained from human blood sera of healthy blood donors. With samples taken from the same serum, the precision of quantitative and qualitative data is very good (not shown). The chromatographic profiles of FAMEs from various human sera of different blood donors show physiological deviations of the relative peak areas, only.

Even cholesterol, as a compound of diagnostic interest in clinical chemistry, can be found and identified in FAME mixtures after TMSH sample preparation. The blood serum profiles of FAMEs prepared with TMSH as reagent correlate very well with serum profiles obtained with other methods [9].

Conclusions

The combined methods of trans-esterification of cell-bound fatty acids by TMSH and of separation of the FAMEs by HRGC are a useful tool for the identification of microorganisms.

The generation of FAMEs by TMSH can be executed directly from serum.

The rapid formation of esters by the TMSH reagent simplifies and accelerates the FAME preparation. The single step method (lysis of microorganisms and trans-esterification) also shows good reproducibility with regard to the patterns of the chromatograms obtained with the FAMEs.

The method has a low detection limit because of the high sensitivity of capillary GC with FID, therefore very small amounts of starting material are required.

All significant types of fatty acid, which may be important for the identification of microorganisms including hydroxy and cyclopropyl fatty acids, can be separated, detected, and identified with highly reproducible relative peak areas.

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