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Journal of Chromatography A, 818 (1998) 133–137

JOURNAL OF CHROMATOGRAPHY A

Short communication

Microwave induced rapid transmethylation of fatty acids for analysis of food oil

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Received 5 March 1998; received in revised form 22 June 1998; accepted 23 June 1998

Abstract

Microwave irradiation enhances the rate of transmethylation of fatty acids in rice bran oil catalyzed by sodium methoxide (1 or 0.3%). The reaction is completed in 40 s in methanol–toluene (1:10, v/v) and in 15 s in methanol–toluene (1:3, v/v). A high concentration of methanol in the mixture raises the temperature rapidly and results in a fast transmethylation. This method gives a yield of fatty acid methyl esters comparable to, or higher than, the traditional preparation and the conditions are mild enough for analysis of oils rich in polyunsaturated fatty acids. The method can rapidly process multiple samples and can be easily applied to the determination of the fatty acid compositions of vegetable oils. The advantages of this new method are its speed, and the accurate results which are comparable to the conventional technique.

Keywords: Vegetable oil; Transmethylation; Fatty acid methyl esters; Sodium methoxide

1. Introduction

Recently, there has been growing interest in applying microwave heating to rapid thermal digestion prior to elemental and chemical analysis of inorganic and biological samples [1]. There have also been increasing efforts to enhance the rate of reactions in organic synthesis [2–5]. The rapid heating by microwave, capable of saving a considerable amount of dissolution time, may eventually replace some of the conventional flame and hot-plate heating protocols [6,7].

The methyl esters of fatty acids are frequently used in gas chromatography (GC) as a reliable method for fatty acid composition analysis [8–13]. Recently, volatile derivatives have also been used in low energy tandem mass spectrometry as the molecular ion to analyze branched-chain fatty acid [14–17] and in thermal analysis of fatty acid compositions [18]. The methyl esters of fatty acids can be prepared by transmethylation of the fat samples either using acidic or basic catalysts [19–25]. Generally, alkali-catalyzed transmethylations proceed under much milder conditions than acidic transmethylations [26,27].

Microwave heating involves direct absorption of energy by functional groups that bear ionic conductivity or a dipole rotation effect, and this energy is then released to the surrounding solution. This absorption of energy causes the functional groups

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0021-9673/98/$19.00
PII: S0021-9673(98)00545-7

involved to have higher reactivity with surrounding reactants than when they are simply incubated with the reactants at the same temperature. Because there is still much debate on the contribution of the nonthermal effects of microwave irradiation, the present study examined the effects of isothermal microwave irradiation on transmethylation of fatty acids in rice bran oil catalyzed by sodium methoxide. The effects of rate-enhancement, the yield of sample recovery, and the prevention of decomposition of polyunsaturated fatty acids during reaction are studied and the results are compared with the traditional method. A practical procedure for applying microwave irradiation is described.

2. Experimental

Toluene was technical grade and redistilled before used. Methanol was obtained from J.T. Baker (USA). Sodium methoxide (30%) in methanol was purchased from Fluka (Switzerland). Heptadecanoic acid (99% purity) was obtained from Sigma (USA). Methyl heptadecanoate was prepared by acid catalyzed esterification. Refined rice bran oil (0.01% free fatty acid) was purchased from Kao Anamai (Bangkok, Thailand). Microwave oven (Amana Radarange model RFS-511, MP 1100 W, USA) with adjustable power level was used for microwave irradiation. GC analysis was carried out on a Shimadzu model 14A gas chromatograph fitted with a split/splitless injector (split ratio 1:50), and equipped with a flame ionization detection (FID) system and a Shimadzu C-R4A data processor. A capillary column (30 m× 0.32 mm I.D. fused-silica bonded with Supelcowax 10) using N_2 as carrier and make-up gases (with flow-rates of 2.5 and 30 ml/min, respectively) was used and separation of fatty acid methyl esters was accomplished isothermally at 170°C. Temperature of injector and detector were set at 230°C.

2.1. Transmethylation

Vegetable oil (1.0 g) and methyl heptadecanoate (300 mg, as internal standard) were dissolved in toluene (100 ml). Transmethylation solution was prepared by mixing the above oil solution (3.0 ml) with a variable volume of methanolic sodium methoxide (1.0 or 0.3%) in a screw-capped tube (16×130 mm) in the case of the heating block or in a custom-made thick-walled PTFE vial (for microwave irradiation; same vials as used in hydrolysis of amino acid on solid-phase resin [28]). For transmethylation, the tube was immersed in a heating block or the PTFE vial was put in the microwave oven for irradiation. After the sample was heated for desired time or irradiated for a certain interval, the reaction was stopped by adding 0.1 ml of acetic acid and the resulting solution was cooled in an ice bath. The organic phase was washed over 1% Na_2CO_3 (3×3.0 ml), distilled water (2×3.0 ml) respectively, and dried over anhydrous sodium sulfate. The esters in organic phase were then analysed by GC.

3. Results and discussion

Methanol and sodium methoxide are polar compounds which adsorb microwaves and release the energy to the surrounding solution efficiently. In contrast, toluene is nonpolar and nearly inert to the microwave irradiation. Toluene not only maintains the solubility of the fatty acids but can also trap released heat from heated methanol and keep the solution cool. Thus, the amount of toluene is normally greater than methanol. Under microwave irradiation for <30 s, neat methanol solution boiled vigorously. Adjustment of the methanol–toluene ratio was required to facilitate transmethylation. Fig. 1 shows the temperature of mixtures of methanol–toluene (1:3 and 1:10, v/v) containing sodium methoxide (1%) after microwave irradiation for different intervals. A high methanol content in the mixture raised the temperature faster. After irradiation for 60 s the temperatures of the mixtures were 56°C and 40°C for the mixtures of ratio 1:3 and 1:10, respectively. In another study the reaction solution containing methanol–toluene (3:1, v/v) was precooled to −5°C and irradiated for 60 s. The temperatures were 25, 40, 48 and 54°C with the irradiated intervals of 15, 30, 45 and 60 s, respectively.

GC was used to monitor the completeness of the reaction in the methylated oil sample. The composition of fatty acid methyl esters were $C_{16:0}$, 23.05%; $C_{18:0}$, 2.49%; $C_{18:1}$, 38.45%; and $C_{18:2}$, 36.01%. Fig. 2 shows the time course for trans-

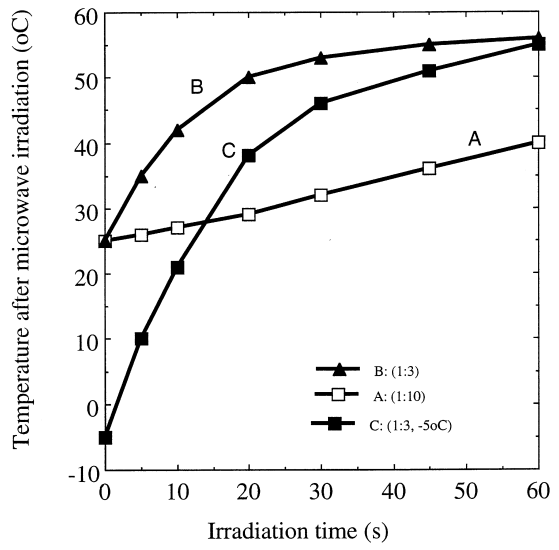

Fig. 1. Temperature of reaction mixture after microwave irradiation. The ratio of methanol–toluene (v/v) in each course curve is (A) 1:10; (B) 1:3; and (C) 1:3 (precooled to −5°C).

methylation in mixtures of methanol–toluene (1:3 and 1:10, v/v) containing sodium methoxide (1.0%, total volume 3.3 ml) under microwave irradiation. The completeness of the transmethylation was calculated from the ratio of peak areas of various fatty acid methyl ester to that of heptadecanoate. The results of the conventional heating under reflux

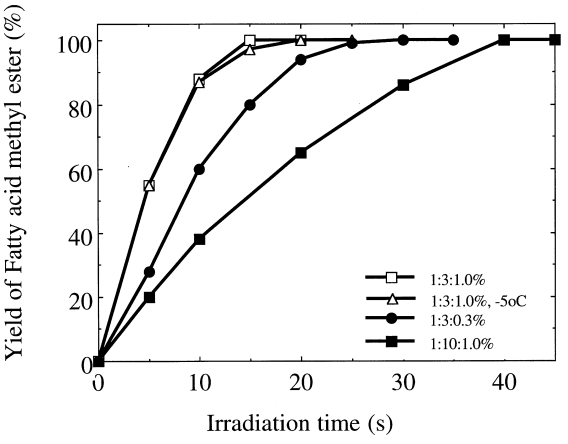

Fig. 2. Yields of fatty acid methyl esters after microwave induced transmethylation of rice bran oil dissolved in a mixture of methanol–toluene–sodium methoxide (v/v/w) in the ratios: (□) 1:3:1.0%; (△) 1:3:1.0% with the reaction mixture precooled to −5°C; (●) 1:3:0.3%; (■) 1:10:1.0%;

conditions were used as reference (100% yield; for example, if the ratio of peak areas of the conventional heating was 10, and the microwave heating for 5 s was 2.5, then the recovery yield of heating for 5 s is 2.5/10×100=25%). Using the heating method, the transmethylation was completely in 2 min when the reaction was carried out under reflux in methanol solution (65°C). It took 5–10 min when incubated at 25°C. Using microwave irradiation, transmethylation was completed in 40 s and the temperature of reaction solution raised from 25 to 38°C in the methanol–toluene (1:10, v/v); whereas in the methanol–toluene (1:3, v/v), the reaction was completed in 15 s, and temperature raised from 25 to 45°C. When the amount of sodium methoxide was decreased from 1.0 to 0.3%, the time required to completed the transmethylation reaction increased from 15 to 25 s. This result shows that a small amount of sodium methoxide (0.3%) as catalyst is sufficient for complete transmethylation of the oil; but the higher amount of catalyst (1%) gives a more consistent result.

Under the microwave irradiation all the transmethylation reaction can be finished within 40 s when the fatty acids are dissolved in different solvent mixtures and catalyzed by different concentration of sodium methoxide (1% or 0.3%),. The temperature of the reaction solution is lower than the reflux temperature (2 min, 65°C). The results show that the microwave irradiation can enhance the rate of transmethylation at least threefold. To find whether the rate-enhancement of transmethylation is due to thermal or microwave irradiation, the sample in the PTFE vial was precooled to −5°C and subjected to microwave irradiation. Results show that the transmethylation proceeded as fast as that started at 25°C, and the reaction was completed in 15 s while the temperature of the solution was raised from −5°C to 25°C. In comparison to the method of incubating at 25°C for 5–10 min to complete the reaction, the microwave irradiation enhanced the rate of transmethylation about 20–40 fold. We reasoned that methanol and sodium methoxide are dipole compounds which absorbed microwave very efficiently and subsequently activate the functional groups to accelerate the reaction. Both transmethylation reactions (started at 25°C and at −5°C) were completed within 15 s under microwave irradiation,

giving direct evidence that microwave irradiation activates the functional group and enhances the rate of reaction. Without microwave irradiation the transmethylation of fatty acids proceed more slowly at low temperatures (<25°C) which makes the microwave effect more significant.

To compare the microwave irradiation procedure with the traditional procedure, transmethylation of vegetable oils (Canola, olive, cottonseed, soybean, sunflower and coconut oils and animal fat) was carried out both by incubation at 25°C for 10 min, and by microwave irradiation for 15 s, and the fatty acid methyl ester compositions determined by GC. Results in Table 1 show that the compositions are comparable. The amount of polyunsaturated oil ($C_{18:2}$:$C_{18:3}$) in each analysis were about the same in all the samples and the differences of relative composition in each oil are negligible. These results shown the microwave irradiation procedure is a reliable transmethylation method.

During the last few years the transmethylation of fat oil catalyzed by sodium methoxide–methanol to offer fatty acid methyl ester derivatives for composition analysis is widely used. It is a requisite that a skilled person carry out the reaction. Therefore, we have attempted a novel approach of utilizing the microwave technology in the rapid preparation of fatty acid methyl ester with the aim of shortening the time of analysis and obtaining data as reliable as those by the conventional method. Use of microwave irradiation for rapid transesterification of lipids and accelerated synthesis of fatty acyl pyrrolidides for analysis by GC–mass spectrometry [29] and low-power focused microwave technology as a new tool for rapid preparation of solid samples for specimen analysis [30] have been documented. Recently, microwave oven with multiple reaction vials (more than 12 vials for gas phase protein hydrolysis and digestion) is commercially available but not for the transmethylation reaction. We used four PTFE vials simultaneously in a microwave oven to prepare the fatty acids sample, the results are consistent with that of a single vial. This results offer encouraging data for further development of the microwave technique with multiple reaction vials for the preparation of fatty acid methyl eater. In conclusion, we believe that the routine fatty acid analysis could be obtained easily and accurately with the introduction of microwave oven-based heating method.

Acknowledgements

This work was supported in part by the National Science and Technology Development Agency, Thailand.

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Table 1
GC analysis of fatty acid compositions of oils prepared by conventional and microwave irradiation methods

Oil	Method[a]	Fatty acid composition (%)						
		C_{14}	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Canola	Conv.	–	4.73±0.04	–	1.91±0.03	61.05±0.25	23.34±0.18	9.02±0.03
	Micro. irra.	–	4.71±0.11	–	1.93±0.04	60.87±0.30	22.61±0.16	9.10±0.19
Olive	Conv.	–	12.93±0.15	1.14±0.05	3.02±0.14	70.69±0.18	12.23±0.09	–
	Micro. irra.	–	12.83±0.18	1.19±0.06	3.53±0.07	70.21±0.19	12.24±0.03	–
Soybean	Conv.	–	11.07±0.09	–	4.26±0.06	22.07±0.07	55.89±0.09	6.70±0.06
	Micro. irra.	–	11.06±0.10	–	4.24±0.12	22.15±0.19	55.71±0.33	6.84±0.23
Sunflower	Conv.	–	6.92±0.06	–	4.42±0.27	17.52±0.02	71.14±0.16	–
	Micro. irra.	–	6.93±0.05	–	4.78±0.08	17.56±0.04	70.74±0.16	–
Animal fat	Conv.	1.61±0.01	26.92±0.11	2.92±0.06	14.23±0.01	43.53±0.03	10.80±0.09	–
	Micro. irra.	1.62±0.09	27.20±0.03	2.97±0.03	13.77±0.10	43.40±0.03	11.03±0.06	–
Rice bran	Conv.	–	23.05±0.16	–	2.49±0.04	38.45±0.17	36.01±0.04	–
	Micro. irra.	–	22.98±0.16	–	2.51±0.03	38.39±0.09	36.13±0.05	–

[a] Conv.=conventional heating method; micro. irra.=microwave irradiation; (all experiments were duplicated).

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