Use of Aqueous HCl/MeOH as Esterification Reagent for Analysis of Fatty Acids Derived from Soybean Lipids

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ABSTRACT

A quick, reliable and very inexpensive method is described for the analysis of fatty acids derived from soybean lipids. The method involves extraction of soybean lipids with petroleum ether, followed by hydrolysis of lipids with KOH/MeOH (0.5 M) for 5 min at 100 C followed by esterification with aq. HCl (36%)/MeOH (4:1, v/v) for 15 min at 100 C. No problems were encountered with the esterification procedure in the presence of water and the procedure gave results comparable to the more conventional BF_3/MeOH reagent. The aq. HCl/MeOH reagent is several hundred times cheaper than BF_3/MeOH, and does not compromise the efficiency of the reagent.

INTRODUCTION

The analysis of lipids and fatty acids is very important in nutritional biochemistry. At present, gas chromatography appears to be the method of choice for the analysis of fatty acids as methyl esters (1). One of the most commonly used esterification reagents is BF_3/MeOH (2-4) and, in spite of the short reaction times (typically 2 min at 100 C), it is an extremely toxic reagent, plus it is expensive and moisture-sensitive. Esterification with anhydrous HCl/MeOH (5) was introduced about 20 years ago, but the procedure was claimed to need vigorously anhydrous conditions. Although a less expensive reagent, H_2SO_4/MeOH, is available (6,7), the procedure involves long reaction times and a more extensive work-up as compared to other reagents such as BF_3/MeOH.

As part of our nutrition program, we wished to develop a quick, inexpensive and reliable method for analysis of several thousand lipids and fatty acid samples extracted from soybeans. The large sample size made the use of BF_3/MeOH unfeasible due to the cost of the reagent. In addition, the use of H_2SO_4/MeOH was not feasible due to long analysis time and extensive work-up. Hence, we had to look for alternative esterifying reagents. In this communication, we report the use of aq. HCl/MeOH as the esterifying reagent.

METHODS

A Varian model 3700 gas chromatograph with a flame ionization detector and a 2-m column of 10% DEGS on Chromosorb was used. The carrier gas was nitrogen at a flow rate of 30 mL/min. The injector and detector were maintained at 260 and 350 C, respectively. The column was operated isothermally at 180 C. The areas of the peaks were calculated by multiplying height × width at half the height. Methanol HCl (36%, d=1,19) and BF_3/methanol (14%) were obtained from Merck and were used without purification.

The following steps were needed for the analysis of fatty acids derived from soybean lipids: (a) extraction—lipids were extracted from soybean with petroleum ether in the conventional way (7); (b) hydrolysis—hydrolysis of the lipids (50 μL) was done with 1 mL of KOH/MeOH (0.5 M) at 100 C for 5 min (8) in 10-in. tightly capped test tubes (see Results and Discussion); (c) esterification—to the hydrolysis mixture was added 400 μL of aq. HCl/MeOH (4:1, v/v) and the mixture was heated in an oil bath for 15 min at 100 C. The tube was cooled and 2 mL of water was added and then extracted with 2 × 3 mL of petroleum ether. The organic layer was dried quickly over anhydrous Na_2SO_4, evaporated and redissolved in 500 μL of $CHCl_3$, and 0.5 μL was used for gas chromatography (GC).

RESULTS AND DISCUSSION

Hydrolysis Step

Haan et al. (9) studied the effect of time and temperature of saponification on serum lipids with tetramethylammonium hydroxide. They noted no ideal conditions in which complete hydrolysis and no loss of polyunsaturated acids occurred together. However, the results for 10 min at 70 C and 90 min at 60 C were found to be satisfactory. Vegetable oils such as soybean are very high in polyunsaturated acids (90%) and are therefore very susceptible to decomposition during lipid hydrolysis. Hence, it was important to determine ideal conditions for lipid hydrolysis with KOH/MeOH.

Temperature for lipid hydrolysis. In order to study the effect of temperature on hydrolysis with KOH/MeOH (1 M), we chose a soybean oil sample at random and performed hydrolysis for 5 min at 4 different temperatures (70, 80, 90 and 100 C) followed by esterification with HCl/MeOH (1:1, v/v) for 2 min at 100 C. Under these conditions, no significant differences were noted in the relative percentage composition of the fatty acids, but the highest response was obtained at 100 C, and hence, we used this temperature for all future hydrolysis.

When we compare our hydrolysis procedure with that of Haan et al. (9), who used tetramethylammonium hydroxide, our procedure seems to be milder, quicker and several times less expensive than theirs. They noted, for example, that when temperature of hydrolysis was raised from 70 to 100 C, the yield of 18:2 was reduced by a factor of 2.5. As 18:2 is the major component ($\simeq$ 60%) of soybean lipids, tetramethylammonium hydroxide would be an unsuitable reagent for lipid hydrolysis. With KOH/MeOH as the hydrolyzing reagent, we noted no such temperature effects. The yield of 18:2 and other fatty acids remained essentially unchanged.

Concentration of hydrolyzing reagent. In order to study the effect of concentration of the hydrolyzing reagent, a soybean oil sample was hydrolyzed for 5 min at 100 C with 5 different concentrations of KOH/MeOH (0.25, 0.5, 1.0, 1.5 and 2.0 M) followed by esterification with aq. HCl/MeOH (1:1, v/v) for 2 min at 100 C. Under these conditions, no significant differences were noted in the relative percentage composition of the fatty acids, but the highest response was obtained with 0.5 M KOH/MeOH solution; hence, we used this concentration for all future hydrolyses.

Time of hydrolysis. In order to study the time needed for hydrolysis, a soybean oil sample was hydrolyzed at 100 C with KOH/MeOH (0.5 M) for different times (5, 10, 20 and 25 min) followed by esterification with aq. HCl/MeOH

(1:1, v/v) for 2 min at 100 C. Under these conditions, no significant differences were noted in the relative percentage composition of the fatty acids, but the highest response was obtained with a 5-min hydrolysis time; thus, we used this time for all future hydrolyses. Harsher conditions such as hydrolysis with KOH (10 M) for 2 hr under reflux (6,7) should be avoided.

Esterification Step

One of the main objectives of this investigation was to use an esterification reagent which would be readily available, offer the economy of H_2SO_4/MeOH and advantages of BF_3/MeOH (i.e., short esterification times). The most obvious esterification reagent was anhydrous HCl/MeOH. However, this reagent has several disadvantages (see Introduction). Hence, we decided to use aq. HCl/MeOH and obtained very good results.

Concentration of esterification reagent. In order to study the effect of concentration of the esterifying reagent, a soybean oil sample was hydrolyzed with KOH/MeOH (0.5 M) at 100 C for 5 min, followed by esterification with 400 μL of aq. HCl/MeOH of varying concentration (4:0.25; 4:1, 4:2 and 4:3, HCl/MeOH, v/v) at 100 C for 2 min. Under these conditions, no significant differences were noted in the relative percentage of the fatty acids, but the highest response was obtained with aq. HCl/MeOH (4:1, v/v). We chose this concentration for all future reactions.

Time of esterification reagent. In order to study the time needed for completion of esterification reaction, we hydrolyzed a soybean sample with KOH/MeOH (0.5 M) at 100 C for 5 min, followed by esterification at 100 C with aq. HCl/MeOH (4:1, v/v) for (2, 5, 10, 15 and 20 min). Under these conditions, the highest response was obtained with a 15-min reaction time, and hence, we used this for all future reactions.

Comparison of aq. HCl/MeOH with BF_3/MeOH. It appeared that aq. HCl/MeOH was functioning adequately and offered all the advantages of BF_3/MeOH, i.e., short reaction time and easy work-up. However, we were not sure if our results were reliable and needed to compare our procedure with results from BF_3/MeOH. We randomly chose 5 soybean oil samples and worked them up as already described, varying only the esterification reagents, and the results are presented in Table I. As can be seen from the unchanged percentage composition of the fatty acids, there was no significant difference between the 2 esterification reagents.

TABLE I

Percentage Composition of the Major Fatty Acids Derived from Soybean Lipids Using Aqueous HCl/MeOH and BF_3/MeOH as the Esterifying Reagents

Sample no.	Esterification reagent	% Composition of major fatty acids derived from soybean lipids[a]			
		C_{16}	18:1	18:2	18:3
1	aq. HCl/MeOH	12.58	21.04	62.05	4.31
	BF_3/MeOH	11.20	19.0	66.24	3.60
2	aq. HCl/MeOH	11.7	19.36	65.35	3.57
	BF_3/MeOH	11.1	18.75	66.39	3.09
3	aq. HCl/MeOH	13.7	20.08	63.54	2.67
	BF_3/MeOH	12.2	20.2	65.12	2.43
4	aq. HCl/MeOH	10.22	24.26	63.52	1.99
	BF_3/MeOH	10.33	24.55	63.00	2.12
5	aq. HCl/MeOH	13.31	24.04	55.02	7.63
	BF_3/MeOH	11.94	23.54	57.23	7.27

[a]About 1% of C_{18} fatty acid was also present.

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