Derivatization of Corn Oil for Analysis by GC

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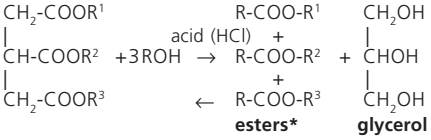
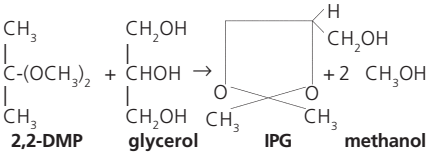
Before the fatty acid composition of a lipid can be analyzed by gas chromatography, the lipid must be converted to low molecular weight, volatile, nonpolar derivatives (e.g., fatty acid methyl esters). This conversion usually is through a transesterification – the glycerol (alcohol) portion of the triglyceride (ester) is displaced by another alcohol, in the presence of an acid. The reaction is represented by the general equation:*



Transesterification is an equilibrium reaction. The stoichiometry of the reaction dictates that for each mole of lipid to be completely derivatized there must be 3 moles of alcohol but, in fact, it is necessary to use an excess of alcohol in the reaction mixture, or to remove one of the reaction products, to drive the reaction to the right. When the second option is employed, the reaction can go to completion.

Transesterification is best done in the presence of a volatile, acidic catalyst■ which can be removed, along with excess alcohol,when the reaction is completed. 2,2-Dimethoxypropane (dimethylacetal acetone/2,2-DMP) also helps drive triglyceride transesterification to completion by reacting with glycerol as it is formed. In the presence of acid, excess 2,2-DMP, and excess alcohol (methanol), glycerol from a lipid transesterification is converted largely to isopropylidine glycerol (IPG) (2). The reaction is:



To ensure complete conversion of the lipid, the amount of 2,2-DMP used should be a molar excess of the total glycerol expected from the reaction.

We used methanolic HCl, 3 N, a general purpose transesterification reagent, to derivatize the triglycerides in a sample of corn oil. We weighed 10 mg of corn oil into reaction vials and added 1 mL methanolic HCl, 3 N▼, 1 mL hexane, and various amounts of 2,2-DMP (0 to 1000 µL) to each vial. The vials were capped and heated at 70 °C for 10-15 minutes. The samples were allowed to cool, then we added 1 mL water and 1 mL hexane. The vials were vigorously shaken and the phases allowed to separate. The ester (upper) layer was sampled for analysis by GC.

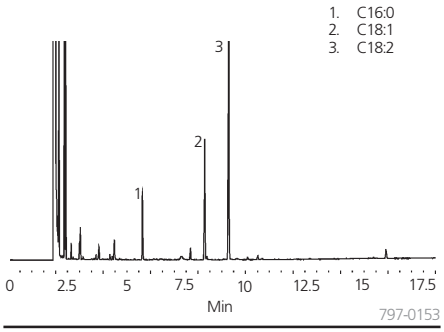
The chromatogram in Figure A is a representative derivatization of corn oil, using methanolic HCl, 3 N, 250 µL 2,2-DMP, and 50 µL dimethylsulfoxide (DMSO). Without 2,2-DMP, transesterification is incomplete. On the other hand, addition of 250 µL of 2,2-DMP causes the reaction mixture to turn yellow,▲ and reaction byproducts appear in the chromatogram. Higher levels of 2,2-DMP produce greater amounts of byproducts. Addition of DMSO to the transesterification reaction mixture inhibits byproduct formation (4). DMSO, however, may interfere with the chromatography of early eluting fatty acid methyl esters (Figure A).

Our study showed that using 2,2-DMP in preparing methyl esters of fatty acids increased the methyl ester yield. Byproducts that can interfere with the chromatography can be eliminated by adding a small quantity of DMSO to inhibit their accumulation.

Figure A. Fatty Acid Methyl Esters from Corn Oil

column: SP-2380, 30 m x 0.25 mm I.D., 0.20 µm film (24110-U)
oven: 150 °C to 250 °C at 4 °C/min.
inj.: 3 µL, split 100:1
det.: FID, 300 °C
carrier gas: helium, 20 cm/sec., set at 150 °C

Derivatized with 1 mL methanolic HCl, 3N, 250µL 2,2-DMP, 50µL DMSO



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Ordering Information:

Description	Cat. No.
Methanolic HCl	
0.5 N, 20 x 1 mL	**33354**
0.5 N, 10 x 5 mL	**33095**
3 N, 20 x 1 mL	**33355**
3 N, 10 x 3 mL	**33051**
3 N, 400 mL	**33050-U**
2,2-Dimethoxypropane	
25 g	**33053**
Corn Oil	
1000 mg	**47112-U**
SP™-2380 Capillary Column	
30 m x 0.25 mm I.D., 0.20 µm film	**24110-U**

References

1. *Bailey's Industrial Oil & Fat Products* John Wiley & Sons (1996).
2. Lorette, N.B., and J.H. Brown, Jr., *J. Org. Chem.,* **24:** 261 (1959).
3. Mason, M.E. and G.R. Walker, *Anal. Chem.,* **36:** 583 (1964).
4. Blau, K. and J.M. Halket (eds), *Handbook of Derivatives for Chromatography* (2nd ed.), John Wiley & Sons (1993) (Cat. No. Z24,622-0).

References 1-3 not available from Supelco.

■HCl is recommended for its high acid strength and because it can be removed easily.

▼Higher concentrations of methanolic HCl greatly reduce the reaction time, but must be stored carefully or they will weaken rapidly. Lower concentrations can be used, but this prolongs the reaction time and requires a greater total amount of reagent (see 3).

▲Produced by a polymer originating from condensation of 2,2-DMP (3).

*Adapted from (1). If alcohol used is methanol, products are methyl esters.