At-line coupling of enzymic sample pretreatment and LC-GC for the *sn*-2 position analysis of edible oils and fats

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Introduction

The stereospecific two position, or *sn*-2 position analysis, provides information on the fatty acids present at the middle position of the glycerol backbone of triacylglycerides (TAGs) in edible oils and fats. Recently, an improved *sn*-2 position method based on scission of the fatty acids on the *sn*-1 and *sn*-3 position with immobilized Lipase D (*Rhizophus Delemar*) was developed [1]. This method consists of several steps and especially the enzymic sample treatment step is time consuming and tedious. Here the enzymic pretreatment step is fully automated and at-line coupled to the LC procedure. In this poster the novel procedure and the results are presented.

Experimental

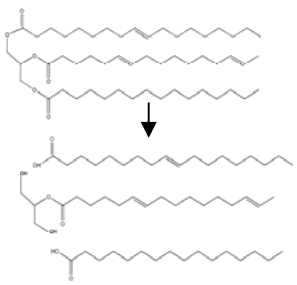

Figure 1: Enzymic hydrolysis of TAGs into FFAs and 2-MAGs.

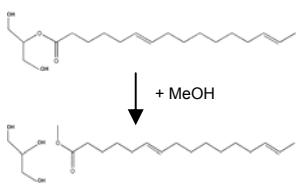

Figure 2: Transesterification of 2-MAGs into FAMEs.

Manual sample preparation

1. ~180 mg oil/fat samples are weighed into 20 ml vials.
2. 6 ml hexane/methanol 98/2 (v/v) is added to each vial.
3. N reactor vials are prepared containing 480 µg silica powder, 700 µl water and 120 mg of the immobilized Lipase D enzyme powder.

Automated enzymic hydrolysis

4. Sample is put in the heated agitator for dissolving the fats/oils.
5. An aliquot of the sample is transferred into the reactor vial.
6. Hydrolysis of the TAGs is performed at 60 °C for 20 minutes (Fig. 1).
7. The mixture is injected into:
   a. LC for automatically collection of the 2-position monoacylglycerols (2-MAGs).
   b. (fast) GC-FID for the determination of free fatty acids (FFA), MAGs, diacylglycerols and TAGs (Fig. 3).
8. LC isolation of the 2-MAGs (Fig. 4).

Future work

9. Evaporation of the fractions collected from the LC to dryness.
10. Transesterification of the fractions containing the 2-MAGs using 10/90 (v/v) mixture of trimethylsulfoniumhydroxide and methyl-*tert*-butyl ether in methanol (Fig. 2).
11. Mixture of fatty acid methylesters (FAMEs) injected into GC-FID (Fig. 5)

Results

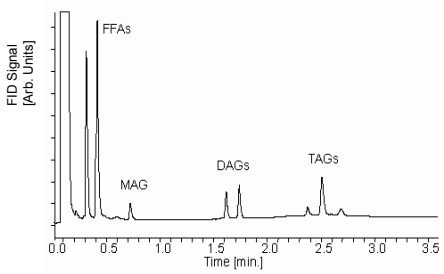

Figure 3: Representative chromatogram of the fast GC quantification of hydrolysis products of the 16:0/18:3/18:0 (PLnS) TAG. GC-column: J&W DB-5HT (3.8 m × 0.32 mm i.d. film thickness 0.1 µm); split injector 400°C, split ratio 1:40; GC-oven: 130°C (0 min), 200°C/min to 365°C (2.65 min); He flow 29 kPa (constant pressure).

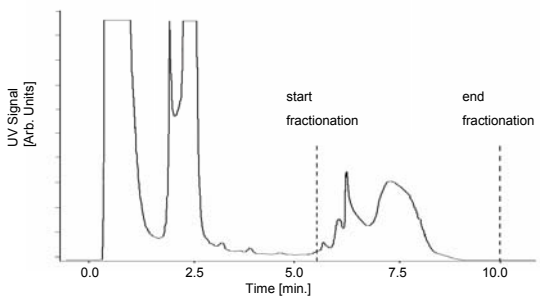

Figure 4: Example chromatogram of the LC isolation of the 2-MAGs. LC-column: Lichrosorb Diol 100 mm × 3 mm i.d., 5 µm particles; mobile phase: isocratic 1.2 ml/min hexane/methanol 98/2 (v/v).

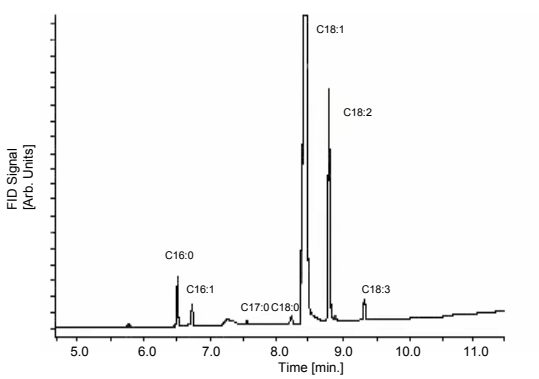

Figure 5: Representative chromatogram obtained in the immobilized lipase D *sn*-2 position analysis for an extra virgin olive oil. GC-column: Varian CP-WAX52CB (10 m × 100 µm i.d. film thickness 0.2 µm); split injector 275°C, split ratio 1:20; GC-oven: 100°C (0 min, 30°C/min to 165°C (0 min), 15°C/min to 255°C (5 min); He flow 350 kPa (constant pressure).

Conclusions

- Automation of the complex procedure for *sn*-2 position analysis of TAGs is possible using simple equipment.
- Manual sample handling is reduced to a minimum which eliminates a potential source of errors, makes the method faster, and also reduces cost of analysis.
- The performance of the new automated method is comparable with that of the manual method.

Reference:

[1] Hans-Gerd Janssen, Karel Hrnčiřík, András Szórádi, Marjolein Leijten, An improved method for *sn*-2 position analysis of edible oils and fats based on immobilized Lipase D (*Rhizopus Delemar*), accepted for publication in J. Chromatography A.