



Analysis of Unsaturated Triglycerides using HPLC

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Food

Abstract

The HPLC method presented here was used to analyze triglycerides, hydroperoxides, sterols, and vitamins with UV-visible diode-array detection (UV-DAD). Spectra were evaluated in order to trace hydroperoxides and to differentiate saturated from unsaturated triglycerides. Unsaturated triglycerides in olive oil have a very distinctive pattern. Other fats and oils are also complex mixtures of triglycerides but exhibit an entirely different pattern. Adulteration with foreign fats and the use of refined triglycerides in olive oil also can be detected through triglyceride analysis.

Sample preparation

Triglycerides can be extracted from homogenized samples with petrol ether. Fats and oils can be dissolved in tetrahydrofuran.¹

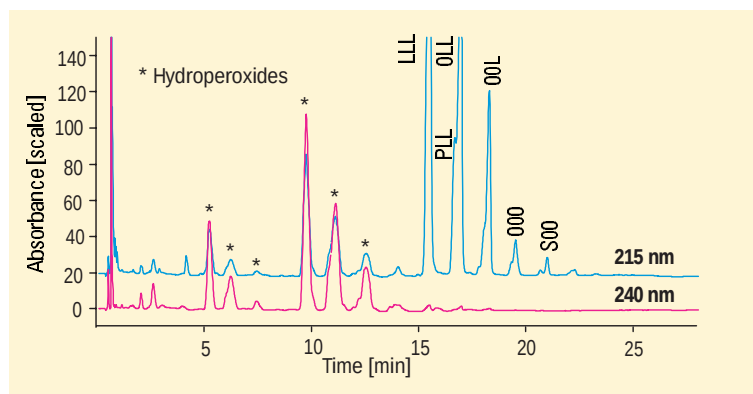


Figure 1
Triglyceride pattern of aged sunflower oil. The increased response at 240 nm indicates hydroperoxides

Conditions

Column

200 ~ 2.1 mm Hypersil MOS, 5 µm

Mobile phase

A = water

B = ACN/methyl-tert.butylether (9:1)

Gradient

at 0 min 87% B; at 25 min 100% B

Post time 4 min

Flow rate 0.8 ml/min

Column compartment 60 °C

Injection vol 1 µl standard

UV absorbance

200 nm and 215 nm to detect triglycerides
240 nm to detect hydroperoxides
280 nm to detect tocopherols and decomposed triglycerides (fatty acids with three conjugated double bonds)

Sample preparation

Samples were dissolved in tetrahydrofuran (THF).



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HPLC method performance

Limit of detection

for saturated triglycerides
>10 µg

for unsaturated triglycerides

fatty acids with 1 double
bond >150 ng

fatty acids with 2 double
bonds >25 ng

fatty acids with 3 double
bonds <10 ng

Repeatability of

RT over 10 runs <0.7 %

areas over 10 runs <6 %

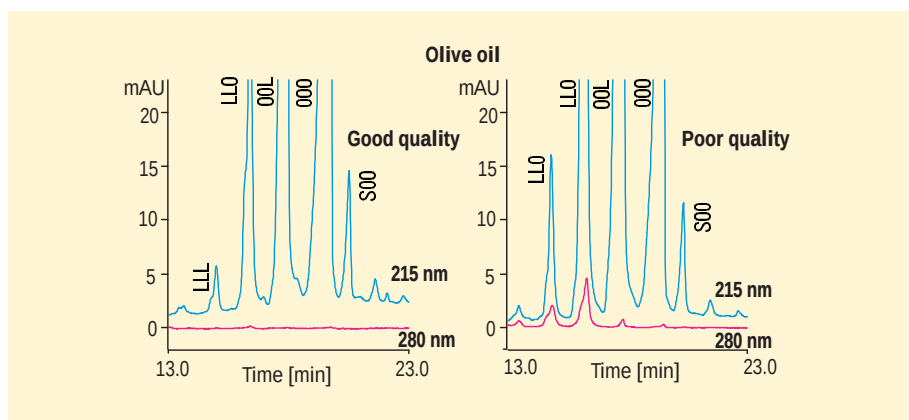


Figure 2

Analysis of olive oil. The response at 280 nm indicates a conjugated double bond and therefore poor oil quality

References

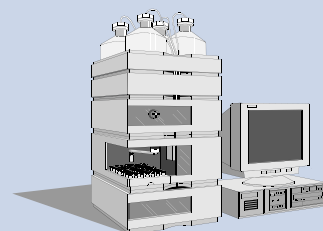
1.

“Determination of triglycerides in vegetable oils”, EC Regulation
No. L248, 28ff.

Equipment

Agilent 1100 Series

- vacuum degasser
- quaternary pump
- autosampler
- thermostatted column compartment
- diode array detector, Agilent ChemStation + software



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