Fresenius Z. Anal. Chem., 307, 115–119 (1981)

Fresenius Zeitschrift für Analytische Chemie

Mass Spectrometric Determination of Triglyceride Patterns of Fats by the Direct Chemical Ionization Technique (DCI)

E. Schulte

Inst. f. Lebensmittelchem., Westfäl. Wilhelms-Univ., Piusallee 7, D-4400 Münster, Federal Republic of Germany

M. Höhn and U. Rapp

Varian GmbH, Postfach 144062, Barkhausenstrasse 2, D-2800 Bremen 14, Federal Republic of Germany

Massenspektrometrische Ermittlung des Triglyceridmusters von Fetten durch Direkte Chemische Ionisation (DCI)

Zusammenfassung. Das Triglyceridmuster eines Fettes läßt sich ohne vorherige chromatographische Fraktionierung auf rein massenspektrometrischem Wege ermitteln. Dabei werden die Triglyceride nach ihrer C-Zahl und der Zahl der in ihnen vorkommenden Doppelbindungen getrennt. Die Probeneingabe in die Ionenquelle erfolgte mit Hilfe einer DCI-Schubstange, die eine schnelle Gesamtverdampfung gestattet. Die chemische Ionisation mit Ammoniak als Reaktantgas ergibt hauptsächlich das Quasimolekularion $(M+NH_4)^+$. Während des Verdampfungsvorganges wurden mit Hilfe eines Datensystems kontinuierlich Massenspektren aufgenommen und diese zu einem einzigen Spektrum aufaddiert, das repräsentativ für die untersuchte Fettprobe ist.

Summary. The triglyceride pattern of fat samples is deduced without prior chromatographic separation exclusively from mass spectrometric data. The separation of the triglycerides is achieved according to their C-number and the number of double bonds. The samples are introduced into the ion source of the mass spectrometer by means of a special DCI sample rod permitting a fast and reproducible total evaporation. The chemical ionization technique using ammonia as reagent gas yields predominantly quasimolecular ions $(M+NH_4)^+$. During the evaporation process mass spectra are continuously acquired with a data system and added up to one spectrum which is representative of the fat sample measured.

Key words: Best. des Triglyceridmusters von Fetten; Chemische Ionisation, direkte

Introduction

To identify and characterize a fat sample or to determine admixtures of other fats, the fatty acid pattern was hitherto used for analysis. However, the most reliable way to procede obviously is to analyse the triglyceride pattern itself.

The analytical separation of triglycerides has been performed by several chromatographic methods, including gas chromatography (GC) with packed or capillary columns, thin-layer chromatography (TLC) on paraffine or $AgNO_3$ containing coatings as well as high pressure liquid chromatography (HPLC) on C_{18}-material or Ag^+ ions containing column packings. All the methods mentioned comprise significant drawbacks such as insufficient separation or low sensitivity or unadequate and indefinitive attribution of components.

The mass spectrometric techniques are important supplements to the chromatographic methods and are able in some aspects to overcome the above mentioned drawbacks. In 1970 Hites [1] already performed MS analysis of triglycerides with a direct inlet system; with a GC/MS combination this type of measurements was reported also [2]. Both papers report data obtained with electron impact ionization (EI), whereby rather molecular ions than fragment ions such as $(M\text{-}RCO_2H)^+$ are produced. The $(M\text{-}RCO_2H)^+$ ions do not permit an unequivocal analysis of triglyceride mixtures.

Murata and Takahashi [3, 4] first reported on measurements of triglycerides by means of the Chemical Ionization (CI) technique as a soft ionization method. They used ammonia as reagent gas and the direct inlet system or the GC as sample introduction system.

To overcome a fractionation effect which is dependent on the chain length, the authors selected one mass spectrum as the most representative one. When this method was applied to natural fats significant differences occurred, e.g. to GC results of other authors. In this paper we will describe an improvement of the analytical techniques.

0016-1152/81/0307/0115/$01.00

Experimental

Instrumentation. Double focusing mass spectrometer MAT 212 (Varian MAT GmbH, Bremen), equipped with a combination EI/CI ion source and coupled to a SpectroSystem MAT 188 (Varian MAT GmbH, Bremen) for data acquisition and evaluation.

DCI introduction rod.

Operating Conditions. Ion source temperature: 200°C; DCI rod: Rhenium wire (0.1 mm diameter) programmed with 5 mA/s up to 1.5 A. Reagent gas: ammonia, 0.4 Torr pressure inside the chem ionization box. Electron energy: 200 eV. Emission current: 0.2 r Accelerating voltage: 3 kV. Resolving power: 1,200 (10% valle

Samples. The pure triglycerides were purchased from Sigma-Cher D-8021 Taufkirchen, FRG. The fats were dissolved either in tolu or CH_2Cl_2 to yield a concentration of about 10%.

Measuring Procedure. 0.5 µl of the solution of triglycerides was pla with a syringe onto the DCI emitter. After evaporation of the solv the sample was introduced into the ion source. The wire was t heated with a given programme and the triglyceride mixture ev orated completely within the range of 200–400 mA. During whole evaporation process mass spectra were repetitively recor with a scan speed of 1 s/decade. A mass range of m/z 300–1,000 covered. By means of the data system all spectra underneath evaporation curve (reconstructed total ion current) were summed and yielded one mass spectrum representative of the comp mixture sample. The complete procedure needs about 10 min at m

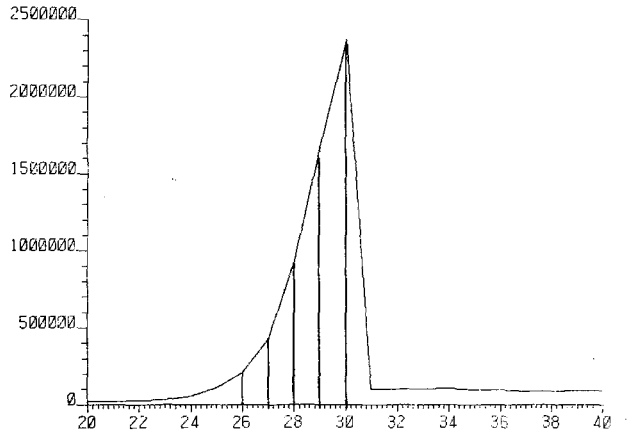

Fig. 1. Total ion current trace, scanned during evaporation of a sample of chocolate fat

Results and Discussion

When using the DCI technique the evaporation tak place from a rhenium wire loop, extended complet into the CI plasma. The term DCI means Dir Chemical Ionization [5] or Desorption Chemic Ionization. The DCI technique has been used in the l few years as another soft ionization technique besid Field Desorption (FD) and "normal" Chemic

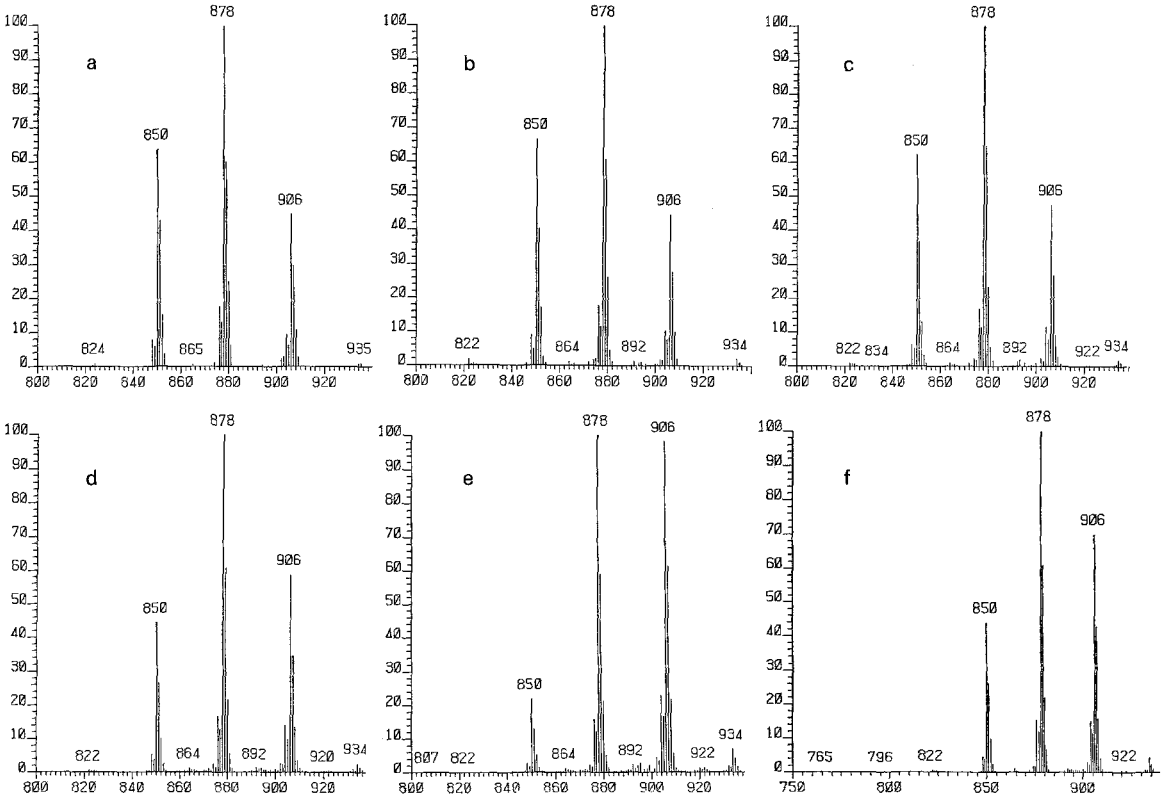

Fig. 2. a–e Single mass spectra of chocolate fat; **f** mass spectrum resulting from addition of **a–e**

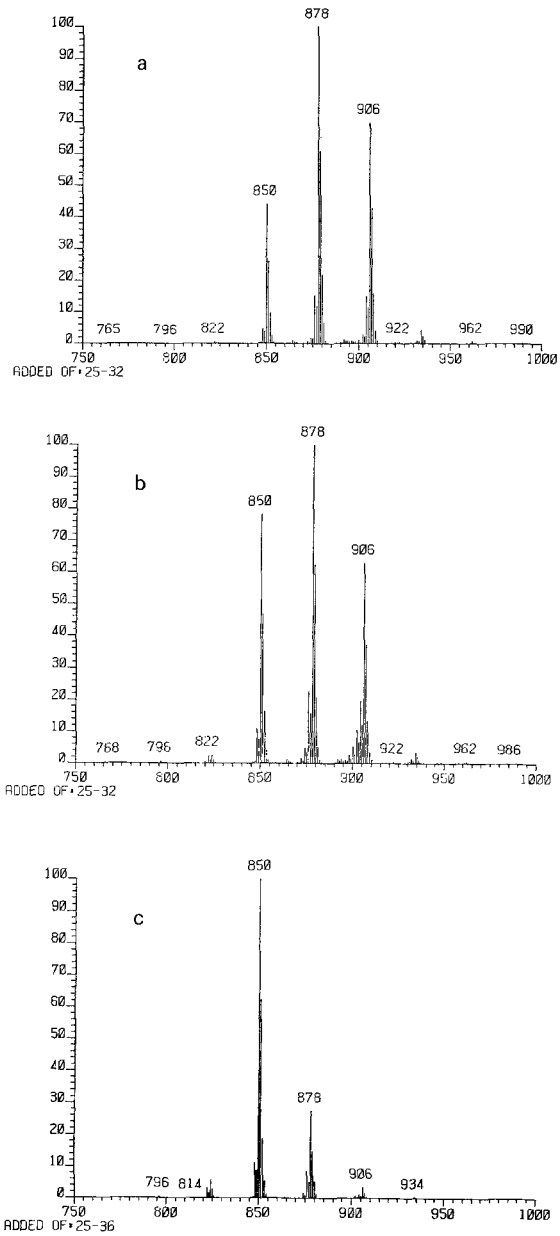

Fig. 3. **a** Same mass spectrum as in Fig. 2*f*. **b** Mass spectrum of a cocoa butter substitute containing chocolate fat. **c** Mass spectrum of a cocoa butter substitute

Ionization. For very different substance classes results were obtained which were up to then only achievable with the field desorption technique [5–8]. When compared to CI, DCI normally shows significantly higher intensities of the quasimolecular ions when measuring thermally labile and polar substances. Furthermore, fragmentation processes are often reduced to a high extent.

During the evaporation of the triglyceride samples a fractionation effect corresponding to the chain length occurs in part. The portion of the higher molecular weight triglycerides increases with time and increased temperature. The use of the classical crucible evaporation system yields a flat curve, resulting in a very long lasting analysis, where one cannot be sure on the completeness of the evaporation process, thus probably resulting in a discrimination of components. Therefore it is important for the measurement of the triglycerides that the heating gradient is such that the evaporation curve is sharp and narrow, to obtain easily a representative mass spectrum.

Figure 1 shows a reconstructed total ion current curve of a fat isolated from chocolate. The vertical lines indicate the position of five single mass spectra displayed in Figure 2a–e. The fractionation effect is clearly visible: *m/z* 850 (corresponding to a C_{50}[1]-triglyceride e.g. oleodipalmitin) has in the first spectrum a much higher intensity than in the fifth one. To yield a representative sepctrum of the total sample, an addition of all mass spectra underneath the evaporation curve is needed. The spectrum displayed in Figure 2f shows the added-up mass spectrum.

The application areas which could be covered by this technique are demonstrated in the following examples. In the first application example (Fig. 3) the DCI spectra are utilized to differentiate fat samples. The mass spectra in Figure 3a and b represent fat samples extracted from two different chocolate samples; Figure 3c shows a spectrum of a commercially available cocoa butter substitute fat derived by fractionation of palm oil.

Figure 3a corresponds to the distribution of the triglycerides in cocoa butter, whereas Figure 3b unequivocally shows a mixture of substitute and natural fat. Most probably, a substitute fat of the type shown in Figure 3c was used, because the group of the C_{50}-triglycerides is significantly increased relative to the C_{52} (*m/z* 878)- and C_{54} (*m/z* 906)-triglycerides [9, 10]. The mass number *m/z* 906 reflects e.g. oleodistearin $(M + NH_4)^+$.

The second application example covers the analysis of more complex fat samples. The triglyceride pattern of butter fat is extremely complex because of the many different fatty acids present in the triglycerides. Hitherto, a complete separation with chromatographic methods sets up extreme difficulties. Figure 4 shows the DCI mass spectra of summer and winter butter.

The well-known characteristic that summer butter contains more unsaturated fatty acids such as oleic acid is clearly indicated. The peak group at *m/z* 710 and 712 as well as *m/z* 682 and 684 corresponding to a C_{40}- or C_{42}-triglyceride shows inversion of the intensity ratios. The triglyceride at mass number *m/z* 710 ows one

1 The number means the sum of the C-atoms of the fatty acids in a triglyceride

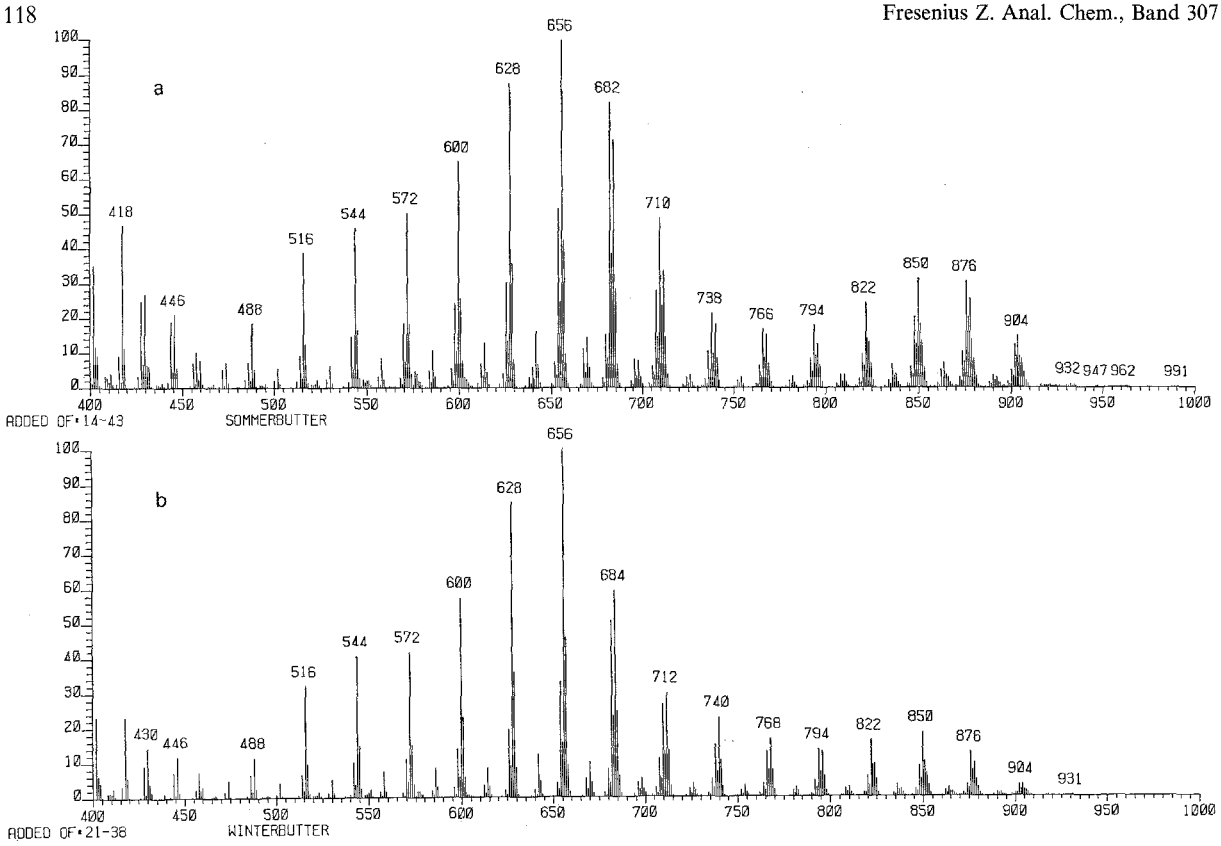

Fig. 4. a Mass spectrum of a summer butter. **b** Mass spectrum of a winter butter

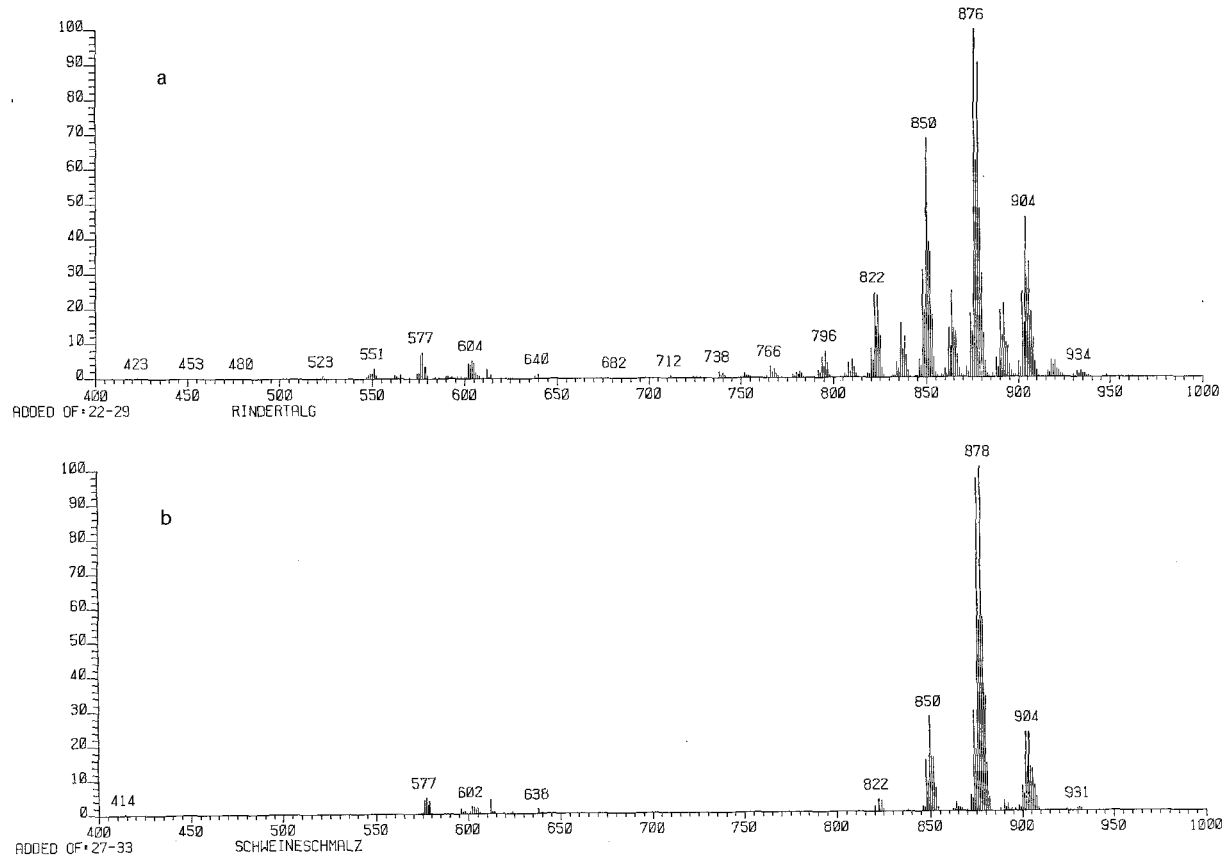

Fig. 5. a Mass spectrum of beef tallow. **b** Mass spectrum of lard

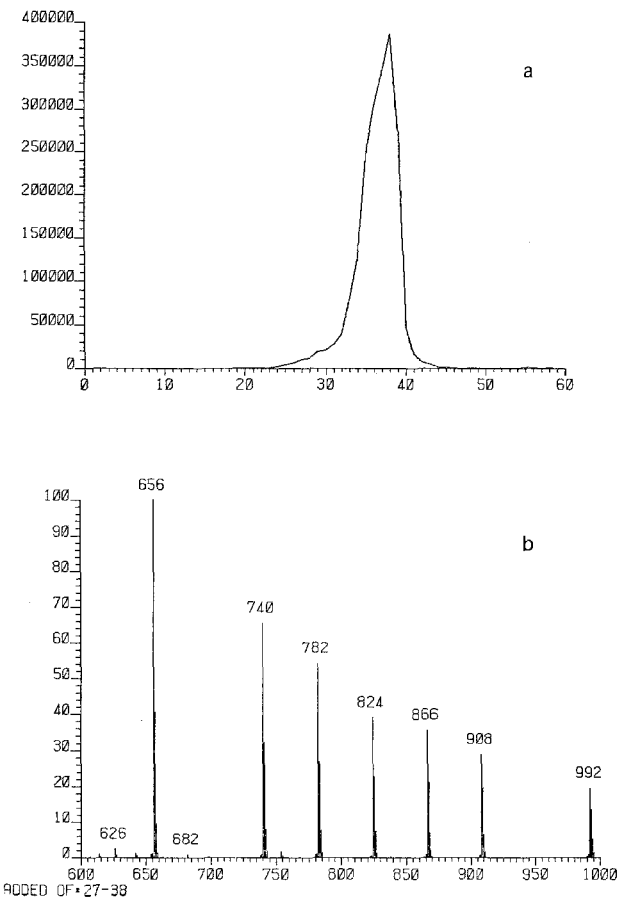

Fig. 6. a Evaporation curve (total ion current trace) of a standard mixture of pure, saturated triglycerides. **b** Mass spectrum of the same mixture. 656 = trilaurin; 740 = trimyristin; 782 = tripentadecanoin; 824 = tripalmitin; 688 = trimargarin; 908 = tristearin; 992 = triarachidin

double bond more than that of m/z 712 which is a completely saturated triglyceride.

The third example once again indicates the capability of the DCI-technique to fingerprint fat samples in a unique manner. The mass spectra of lard (Fig. 5a) and beef-suet (Fig. 5b) show this instructively. The main peak group at m/z 876 and 878 corresponds to a C_{52}-triglyceride with one, respectively, two double bonds in the side chains (e.g. palmitooleostearin and palmitodiolein). Furthermore, it is very nicely documented that ruminants produce significant amounts of odd chain-length fatty acids resulting in peaks at m/z 890/892 or m/z 918 and 920 characteristic of C_{51}- or C_{53}-triglycerides.

Besides the fingerprinting of samples as we have shown above, the quantitative analysis of triglycerides is a crucial point of interest. Measurements performed for calibration purposes yielded up to now the following results. The response of particular triglycerides is decreasing with increasing C-number of the triglyceride. Figure 6 shows the result of a standard test mixture ranging from a C_{36}-(trilaurin) to a C_{60}-(triarachidin)triglyceride and containing the same quantity of each component.

The response is nearly independent from the number of double bonds. Here, the saturated C_{54}-triglyceride (tristearin) m/z 908 $(M + NH_4)^+$ was measured together with triolein (3 double bonds) m/z 902, trilinolein (6 double bonds) m/z 896 and trilinolenin (9 double bonds) m/z 890.

The reproducibility of double or triple measurements turned out to be so promising that exact quantitative evaluations are possible.

In a subsequent publication we will deal mainly with the quantitative aspects in terms of standard deviation and calibration procedures. Furthermore, besides the pure C-number the extent of unsaturation of the triglycerides will also be taken into consideration.

References

1. Hites, R. A.: Anal. Chem. **42**, 1736 (1970)
2. Murata, T., Takahashi, S.: Anal. Chem. **45**, 1816 (1973)
3. Murata, T., Takahashi, S.: Anal. Chem. **49**, 728 (1977)
4. Murata, T.: Anal. Chem. **49**, 2209 (1977)
5. Baldwin, M. A., McLafferty, F. W.: Org. Mass Spectrom. **7**, 1353 (1973)
6. Hunt, D. F., Shabanowitz, J., Botz, F. K., Brent, D. A.: Anal. Chem. **49**, 1160 (1977)
7. Arpino, P. J., Devant, G.: Analusis **7**, 348 (1979)
8. Rapp, U., Meyerhoff, G., Dielmann, G.: Österr. Chem.-Ztg. **81** (4),(1980)
9. Schulte, E., Fincke, A.: GDCh-Hauptversammlung München, Sept. 1977, ref. in Mitteilungsbl. GDCh Fachgr. Lebensmittelchem. Gerichtl. Chem. **32**, 15 (1978)
10. Fincke, A.: Dtsch. Lebensm.-Rundsch. **76**, 162 (1980)

Received December 1, 1980