The Analysis of Triglycerides in Edible Oils by APCI LC / MS

Application

Food

Author

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Abstract

Triglycerides are the major components in edible oils. This application describes a method for analyzing intact triglycerides by LC / MS and the type of information available.

Introduction

Triglycerides are found in both plant oils and animal fats. Their characterization is important for nutritional reasons, where the amount of unsaturation must be known, as well as for determining other characteristics of the oil, such as product purity, suitability for deep-frying, etc.

Traditionally, they have been analyzed by hydrolyzing the triglycerides to yield the fatty acids, which are then derivatized and analyzed as methyl esters by GC/MS [1]. This offers only indirect analysis of the original triglycerides. Triglycerides can be chromatographed intact by HPLC but their lack of a chromophore unless conjugated makes detection difficult [2]. In addition, in order to identify each individual triglyceride in an oil, a larger number of standards than is practical would be required and the analysis time would be quite lengthy.

LC/MS analysis offered the ease of an LC separation plus the specificity of mass detection. Due to the non-polar nature of the molecules, atmospheric pressure chemical ionization (APCI) was employed.

Experimental

The system included an 1100 Series binary pump, vacuum degasser, autosampler, thermostatted column compartment, and LC/MSD. The LC/MSD was used with the APCI source. Complete system control and data evaluation were done on the ChemStation for LC/MS. Earlier work to demonstrate feasibility was done on the 1090 HPLC and 5989B MS Engine equipped with the 5987A Electrospray Accessory and the G1075A APCI source.

A variety of vegetable oils as well as animal fat products such as butter was purchased at local supermarkets. All products were dissolved in isopropanol at a concentration of 20 µL of oil per 10 mL of IPA or 20 mg of fat per 10 mL IPA. Triglyceride standards were purchased from Sigma and prepared by dissolving 10 mg of standard in 5 mL of chloroform then further diluted to the desired concentration with IPA.

Results and Discussion

Separation and Ionization

In order to have the nonpolar triglycerides elute in a reasonable mobile phase composition, a Hypersil MOS (C8) column was used. Even this column required using isopropanol and n-butanol to elute the larger triglycerides. The final gradient was able to resolve cis and trans isomers of C18:1 triglyceride standards as shown in Figure 1.

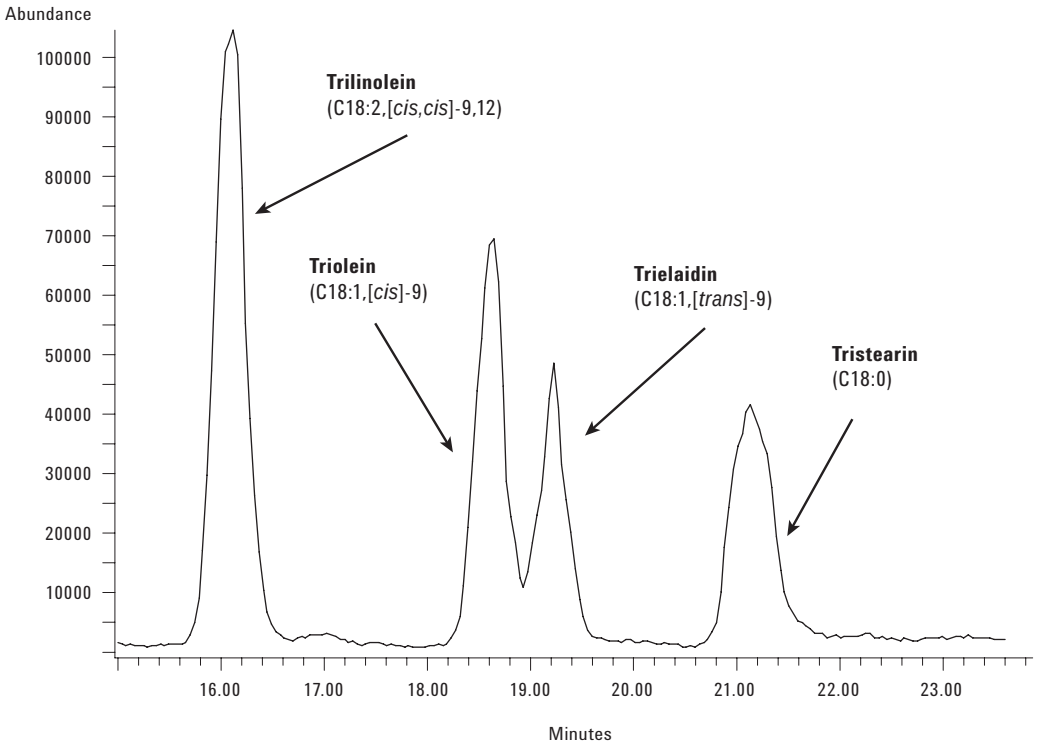

Figure 1. TIC showing separation obtained for four C18 triglyceride standards. Note that *cis* and *trans* isomers are well resolved.

LC Conditions

Column:	Hypersil MOS, 200 mm × 2.1 mm, 5 µm
Mobile phase:	A = 60:40 water:isopropanol + 25 mM ammonium formate B = 10:10:80 water:isopropanol:n-butanol + 25 mM ammonium formate
Gradient:	Start with 30% B at 1.5 min 30% B at 25 min 60% B at 28 min 100%B
Flow rate:	0.25 mL/min
Column temperature:	50 °C
Injection volume:	1.5 µL

MS Conditions

Source:	APCI
Ion mode:	Positive
V_{cap} voltage:	4000 V
Nebulizer:	50 psig
Drying gas flow:	4 L/min
Drying gas temp:	325 °C
Corona:	4 µA
Vaporizer temperature:	300 °C
Scan range:	300-1100 *m/z*
Step size:	0.1 *m/z*
Peak width:	0.3 min
Time filter:	On
Fragmentor:	80 V

It was found that the addition of ammonium formate to the mobile phase allowed the formation of an ammonium adduct $[M+18]^+$ that was more stable than the protonated $[M+1]^+$ species formed without the ammonium formate. The effect of adding ammonium formate is shown in Figure 2. Additionally, the vaporizer had to be lowered to 300 °C to minimize fragmentation and enhance the molecular signal. If structural information was desired, in-source collision induced dissociation (CID) was employed by raising the fragmentor from 80 volts to 150 volts.

Qualitative Spectral Information

Under these analytical conditions, each triglyceride shows predominantly an M+18 adduct ion and few other major fragments. Since triglycerides in plants are made up predominantly of fatty acids with even numbers of carbons (C12, C14, etc.) and either 0, 1, or 2 double bonds per acid, much can be determined from this single adduct ion. A spreadsheet was created to determine the possible combinations of double bonds and carbon chain length. This was determined from the basic formula:

$$M = 218.03 + 28.03*m + 26.02*n$$

Where M is the mass of the observed adduct ion, m is the number of ethylene ($–CH_2–CH_2–$) groups and n is the number of ethenyl (–CH=CH–) groups. Since only integer solutions are allowed, the number of possible answers is typically very short. The choice is reduced even further if you assume fatty acids of the same length and unsaturation as the most likely solution, followed by only small differences in the fatty acids. For example, an unsaturated butyric

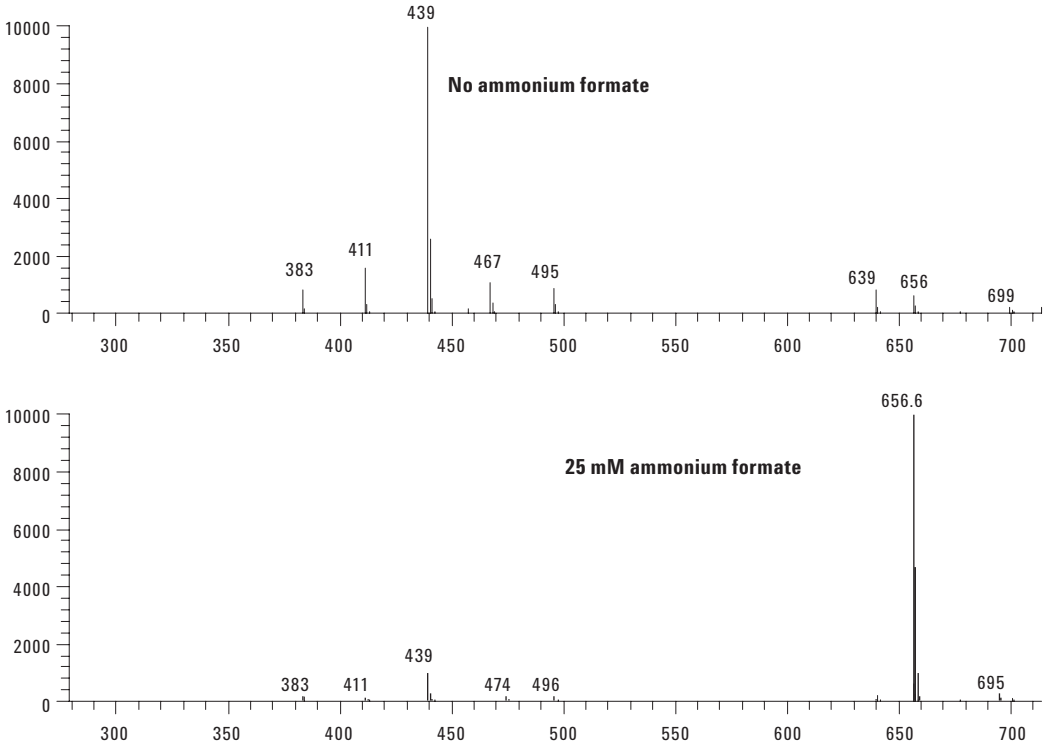

Figure 2. Effect of ammonium formate on the spectrum of trilaurin from coconut oil.

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Column:	Hypersil MOS, 200 mm × 2.1 mm, 5 µm
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MS Conditions

Source:	APCI
Ion mode:	Positive
V_{cap} voltage:	4000 V
Nebulizer:	50 psig
Drying gas flow:	4 L/min
Drying gas temp:	325 °C
Corona:	4 µA
Vaporizer temperature:	300 °C
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acid with two oleic acids is not a likely solution. Figure 3 shows a typical TIC trace for coconut oil with the masses of the base ion annotated over each peak. Inserting these masses into the spreadsheet produces the results shown in Table 1. Further confirmation, if needed, can be obtained using in-source CID. This is shown in Figure 4.

Most oils do not produce as simple a TIC trace as coconut oil. Figure 5 shows a trace for two grades of olive oil. Some differences are visible in the TIC but not much useful information is apparent. However, by looking at extracted ion chromatograms (EICs), much more can be determined. Figure 6 shows the results from extracting the signals for *m/z* 908.8, 906.8, 904.8, 902.8, 900.8 and 898.8. These correspond to triglycerides made up from 3 C18 fatty acids with 0, 1, 2, 3, 4, and 5 double bonds, respectively. Not surprisingly, the dominant ion is at *m/z* 902.8, which corresponds to the ammonium adduct ion for triolein (3 C18:1). Oleic acid is the major fatty acid component in olive oil. Using EICs in this way, different oils can be compared to see how they differ in degree of unsaturation.

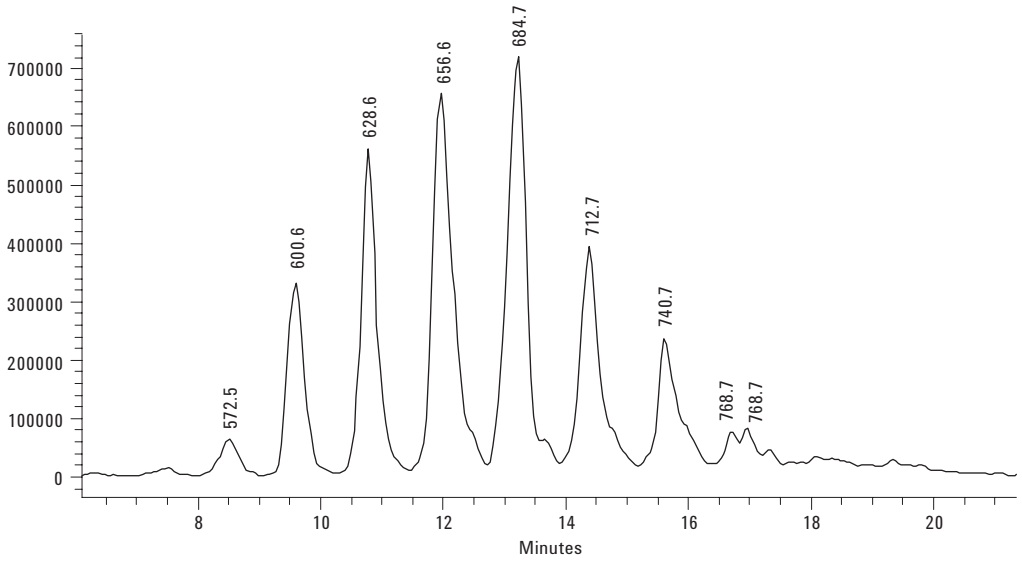

Figure 3. TIC showing mass of the base peak of the spectrum from peak apex. Peaks differ by *m/z* 28 corresponding to difference in the number of –(CH_2CH_2)– groups on sidechains.

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Nebulizer:	50 psig
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Table 1. Structure Calculations for the Major Peaks in Coconut Oil

Observed ion	Calc. MW	Backbone removed	m	n	Possible sidechains	No. double bonds
516.50	498.52	280.42	10	0	C8, C8, C10	0
544.55	526.52	308.42	11	0	C8, C10, C10 or C8, C8, C12	0
572.55	554.52	336.42	12	0	3 × C10	0
600.55	582.52	364.44	13	0	C10, C10, C12	0
628.55	610.52	392.44	14	0	C10, C12, C12 or C10, C10, C14	0
656.70	638.67	420.59	15	0	3 × C12	0
684.75	666.72	448.64	16	0	C12, C12, C14	0
712.75	694.72	476.64	17	0	C12, C14, C14 or C12, C12, C16	0
740.80	722.77	504.69	18	0	3 × C14	0
768.75	750.72	532.64	19	0	C14, C14, C16	0
796.75	778.72	560.64	20	0	C14, C16, C16 or C14, C14, C18	0

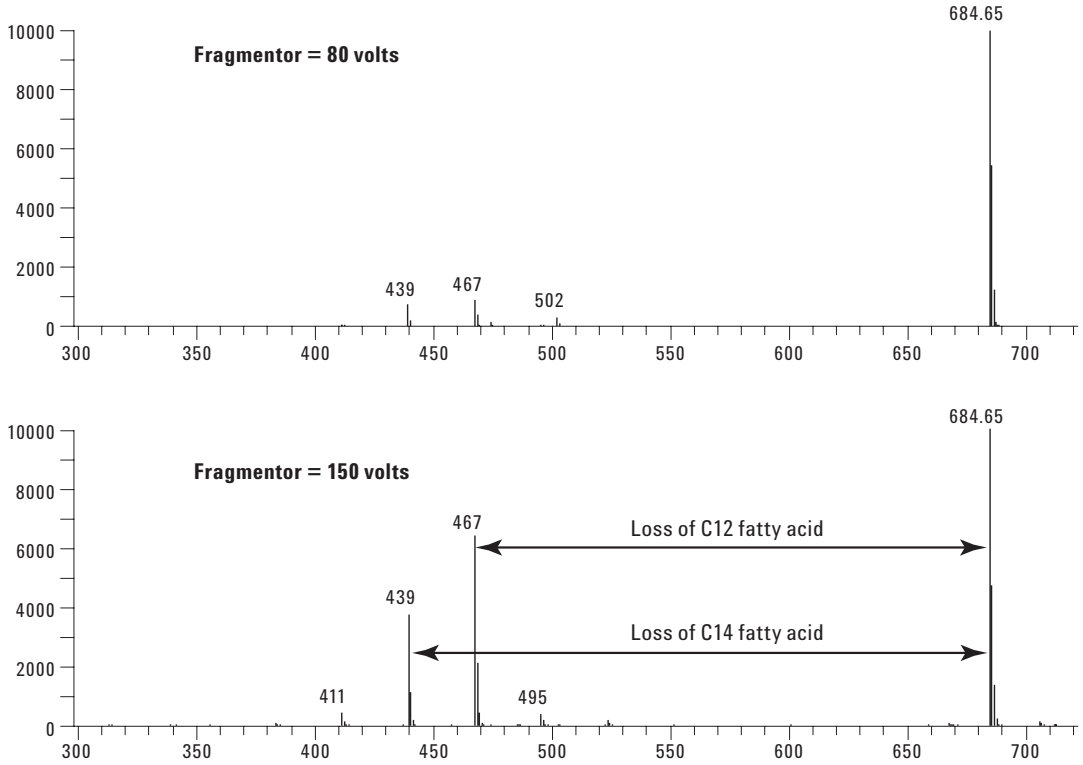

Figure 4. The effect of raising the Fragmentor voltage on a mixed triglyceride in coconut oil. The fragment masses and ratios are consistent with two C12 fatty acids and one C14.

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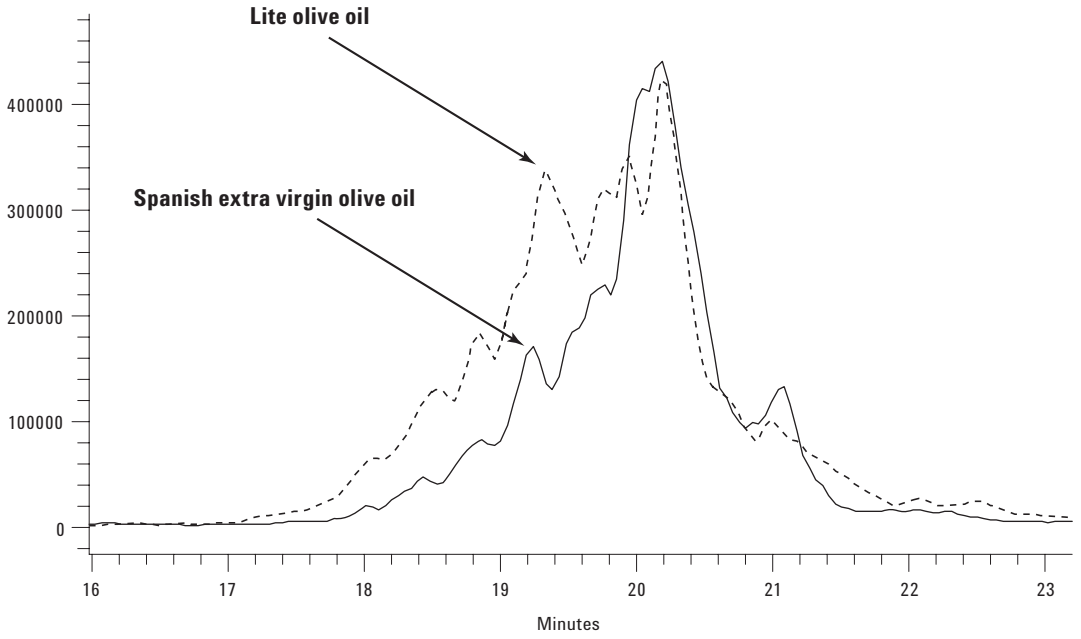

Figure 5. The TIC of lite and Spanish extra virgin olive oils. The data indicate lite oil is more unsaturated or has shorter fatty acids. Further examination of EICs indicates more unsaturation.

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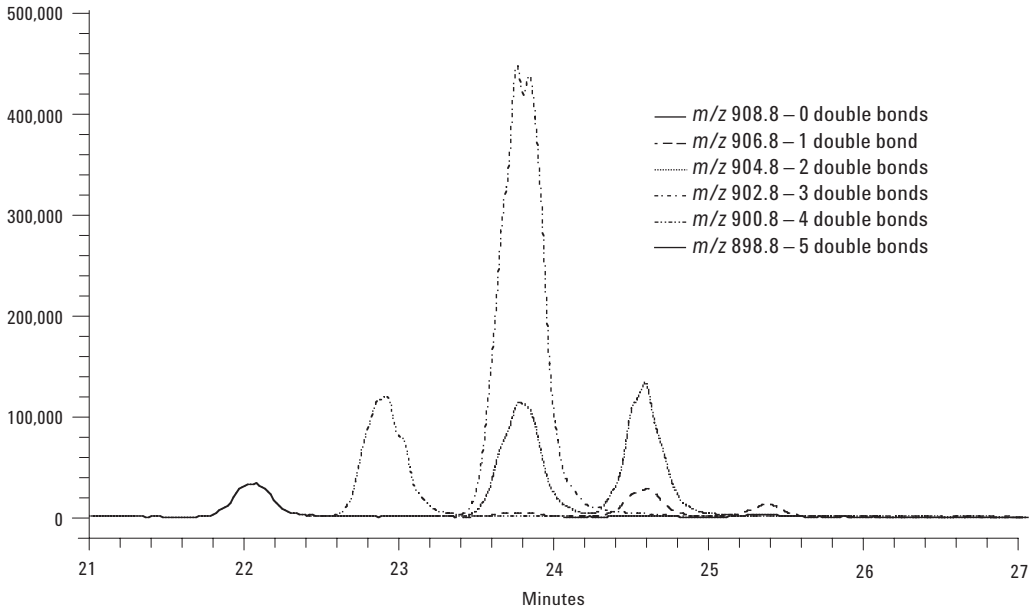

Figure 6. Extracted ion chromatograms (EICs) for olive oil corresponding to the 3 C18 series. Triolein is the major component.

Conclusions

LC/MS using APCI as an ionization process provides a simple way to analyze oils and fats for triglycerides. Information on the type of fatty acids, including the degree of unsaturation, can be obtained directly rather than indirectly. This information can be used for determining nutritional value and other physical properties of the oil or fat. It also has the potential of determining the amount of processing the oil has undergone (extra virgin versus pure). Little or no sample cleanup is required, allowing for a rapid analysis.

References

1. M. M. Mossoba and D. Firestone, "New Methods for Fat Analysis in Foods," *Food Testing and Analysis*, **1996**, 2 (2), 24–32.
2. R. Schuster, "Multicomponent Analyses of Fats and Oils Using Diode-Array Detection," Hewlett-Packard Application Note, **1987**, publication number 59546269.

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