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Application News

Gas Chromatography Mass Spectrometry No.M248

Qualitative Analysis of Triglycerides in Cooking Oil Using GC/MS

Cooking oil is primarily composed of a mixture of triglycerides, which are three fat acids molecules attached to a glycerol molecule by ester linkages. There are many naturally occurring triglycerides, but few of these are included in commercial mass spectral databases, making the identification of the compounds by library searches difficult. When these compounds are analyzed by electron ionization (EI), the molecular ion is often not detected due to the extensive fragmentation of these compounds. Analysis of the compounds by both EI and chemical ionization (CI) provides significantly more information for positive compound identification. CI dramatically increases the intensity of the molecular ion, and the combination of the molecular weight information from CI, and the fragmentation information from EI enhances positive identification of the triglyceride fatty acid composition. The following examples illustrate the use of the combined data from EI and CI for the triglyceride analysis of a butter sample by GC/MS.

■ Analytical Conditions

Commercial butter was diluted to about 5% with chloroform, and then injected into the GC/MS. The analytical conditions used for the analysis are shown in Table 1.

Table 1 Analytical Conditions

Model	: GCMS-QP2010 Plus			-- EI Mode --	
				Ionization Method	: EI
-GC-				Scan Range	: *m/z* 29 - 1090
Column	: Frontier Labs UA$^+$-65 (30 m × 0.25 mm I.D. df = 0.1 µm)			Scan Interval	: 0.5 s
Column Temp.	: 150 °C (1 min) -5 °C/min-350 °C (20 min)			-- CI Mode --	
Carrier Gas	: He (Constant Linear Velocity Mode)			Ionization Method	: CI
Linear Velocity	: 45 cm/s			Scan Range	: *m/z* 500 - 1090
Injector Temp.	: 370 °C			Scan Interval	: 0.5 s
Injection Method	: Split	-GC/MS-		Reagent Gas	: NH_3
Split Ratio : 50:1	: 50:1	Interface Temp.	: 350 °C		
Injection Volume	: 0.5 µL	Ion Box Temp.	: 250 °C		

■ Measurement Results - Total Ion Chromatogram

The diluted butter sample was analyzed by EI and CI using the instrument conditions in Table 1. Only the total ion chromatogram (TIC) by EI is shown in Fig. 1, and the TIC by CI is essentially identical to that by EI.

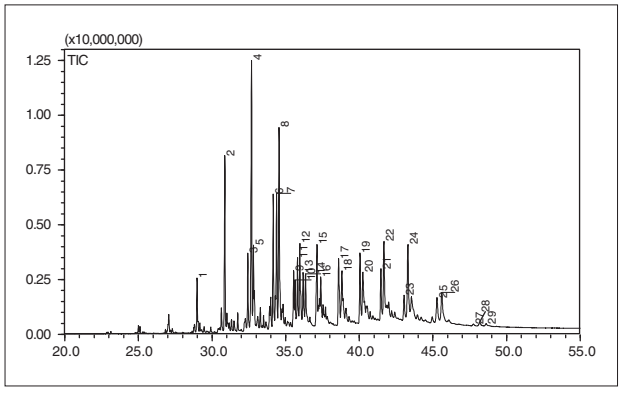

Fig.1 TIC of Butter Oil

■ Structural Analysis

All triglycerides have the same basic structure which is shown in Fig. 2, with three fatty acid molecules bonded to a molecule of glycerol. Triglycerides can be divided into three subgroups, based on the fatty acids bonded to the glycerol. The first group has three fatty acids of the same molecular weight, the second group has three fatty acids that all have different molecular weights, and the third group has two fatty acids with the same molecular weight and the third acid has a different molecular weight.

When CI is utilized to determine the molecular weight of a compound, the molecular ion is usually not detected; instead a pseudomolecular ion (M+x) is detected, and the molecular weight (M) can be calculated from this ion. When a compound is ionized in CI mode either a proton or a molecule of the reagent gas with a proton is attached to the molecule. Ammonia is used as the reagent gas, so the pseudomolecular ion will be the molecule with a proton or an ammonium ion attached, so the ion detected will be either M+1 or M+18.

Fig. 3 shows a typical EI mass spectrum.

The ions formed by the loss of the fatty acids from the molecule are easily identified in the high mass region of the EI mass spectrum. Because the ions that correspond to the molecular weight are not easily identified in the EI mass spectrum, it is difficult to determine the composition of the triglycerides. By using the molecular weight of the triglyceride determined from the CI mass spectrum using ammonia and the masses corresponding to the loss of fatty acids in the EI mass spectrum, the fatty acids in the triglyceride can be identified.

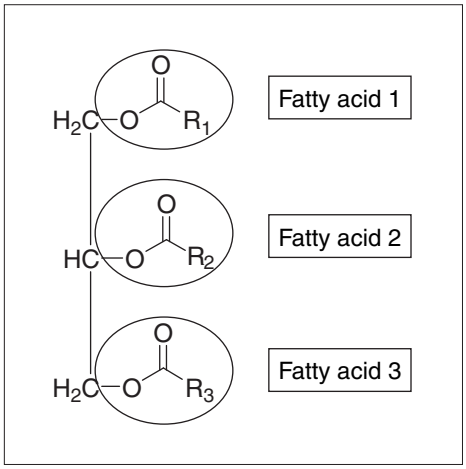

Fig.2 Structure of Triglyceride

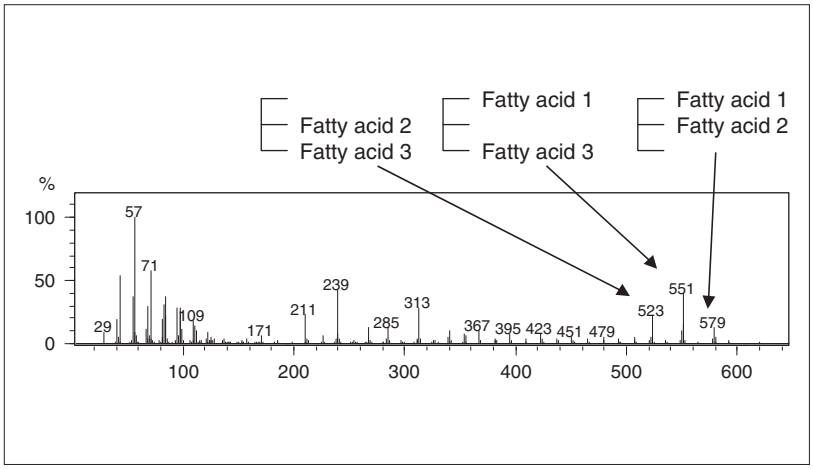

Fig.3 EI Mass Spectrum of Typical Triglyceride

Combination of EI with CI

From the chemistry of the triglycerides and the fatty acids, it is known that the triglycerides will add an ammonium ion during CI using ammonia, so the ions detected are the molecular weight plus 18, the mass of the ammonium ion. It is also known that the fragments generated by the loss of the fatty acid groups consist of the deprotonated fatty acid ion, so the molecular weight of the fatty acid is the mass of the ion plus 1, the mass of a proton. Using these rules triglyceride can be analyzed using the following expressions.

Expected molecular weight of triglyceride = Ammonia CI ion of interest - 18
Expected molecular weight of fatty acid = Expected molecular weight of triglyceride - EI ion of interest + 1

The mass spectra of the triglycerides can be classified into to 3 patterns. One pattern is one in which the 3 fatty acids have the same molecular weight, another is where all 3 have different molecular weights, and the third is where 2 are the same and the 1 is different. Following is an explanation of the analytical procedure using both the CI and EI mass spectral data using mass spectra corresponding to the respective patterns taken from the analysis of the butter sample.

■ Pattern 1

Fig. 4 shows the EI mass spectrum of peak 29 corresponding to the pattern in which the molecular weights of the 3 fatty acid molecules of the triglyceride are the same. Here it is noteworthy that one ion peak is detected in the range of mass 500 to 650. The CI mass spectrum of peak 29 is shown in Fig. 5. Since the detected 902 ion is presumed to be an ammonium ion adduct (+18), the molecular weight of the triglyceride is expected to be as calculated below.

Molecular weight calculation for peak 29: 902 - 18 = 884

From this, the molecular weight of peak 29 is presumed to be 884. Next, the fatty acid composition is predicted by combining the expected molecular weight obtained from CI with the ion of interest 603 obtained by EI.

Molecular weight calculation for peak 29 fatty acid: 884 - 603 + 1 = 282

The molecular weight of the fatty acid derived from the above calculation is presumed to be 282. Therefore, the fatty acid in the triglyceride of peak 29 is presumed to have a carbon number of 18, and contains 3 fatty acid molecules each with 1 double bond.

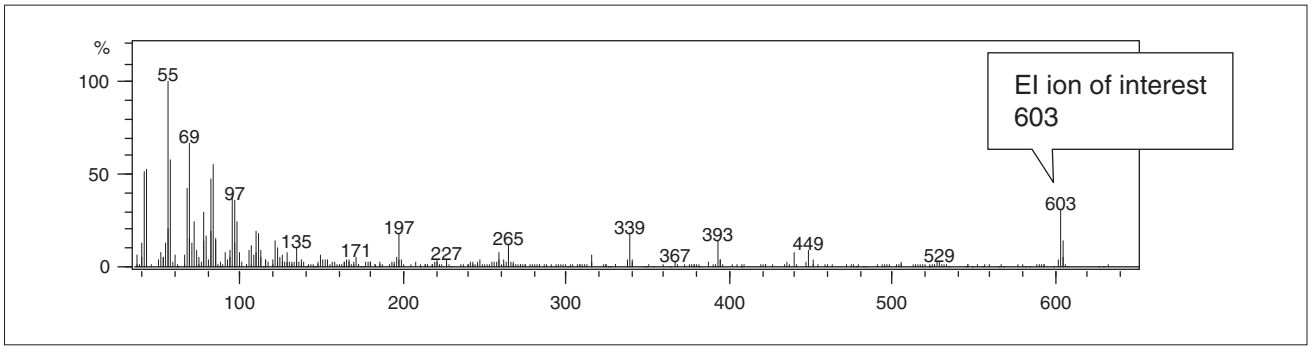

Fig.4 EI Mass Spectrum of Peak #29

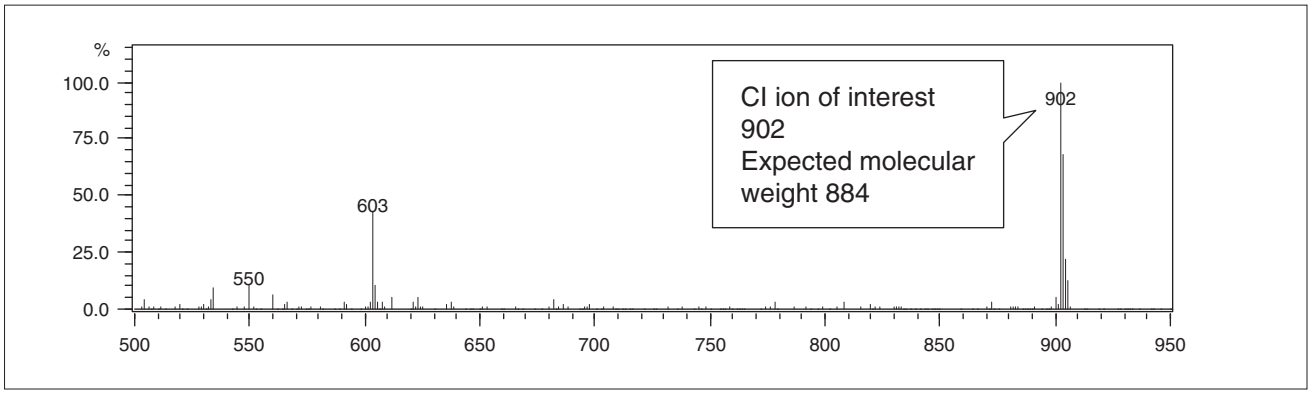

Fig.5 CI Mass Spectrum of Peak #29

■ Pattern 2

Fig. 6 shows the EI mass spectrum of peak 21 corresponding to the pattern in which the molecular weights of the 3 fatty acid molecules of the triglyceride are the all different. Here the distinction is that 3 ion peaks (523, 551, 579) are detected in the range of mass 500 to 650. Three separate peaks are seen because the 3 molecular weights of the triglyceride fatty acids are all different. The CI mass spectrum of peak 21 is shown in Fig. 7. The 3 different fatty acid molecules will be referred to as fatty acid 1, 2, and 3.

Molecular weight calculation for peak 21: 824 - 18 = 806
Molecular weight calculation for peak 21 fatty acid 1: 806 - 523 + 1 = 284
Molecular weight calculation for peak 21 fatty acid 2: 806 - 551 + 1 = 256
Molecular weight calculation for peak 21 fatty acid 3: 806 - 579 + 1 = 228

From the above calculations, the molecular weights of the 3 fatty acids are presumed to be 284, 256, and 228. The triglyceride of peak 21 is presumed to be composed of C18, C16, and C14 saturated fatty acids

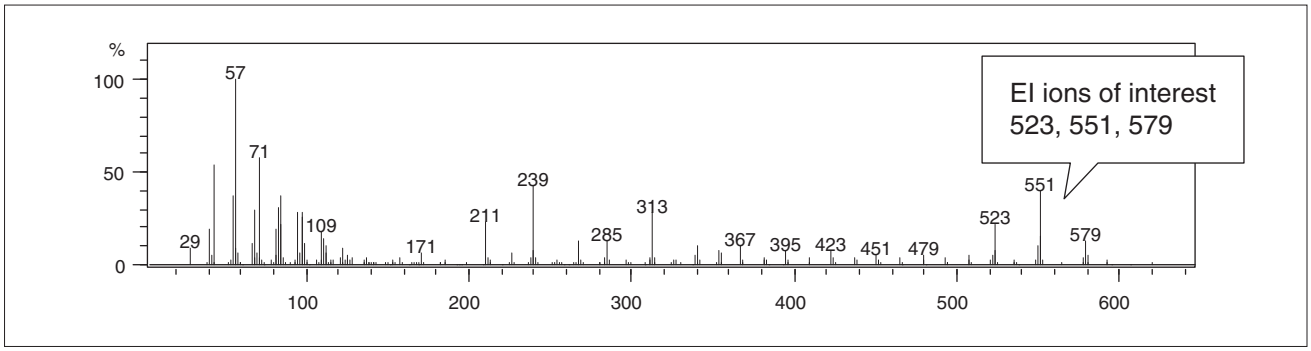

Fig.6 EI Mass Spectrum of Peak #21

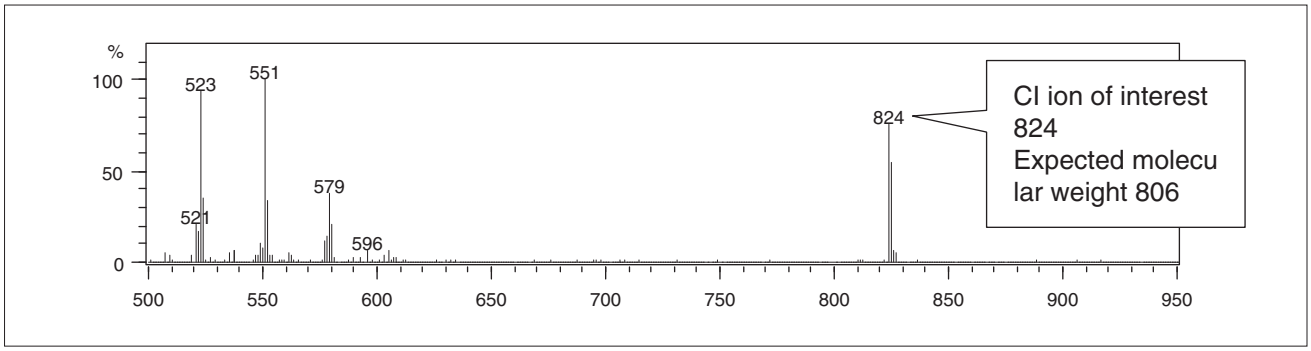

Fig.7 CI Mass Spectrum of Peak #21

■ Pattern 3

Fig. 8 shows the EI mass spectrum of peak 19 corresponding to the pattern in which the molecular weights of 2 fatty acids are the same, and one is different. Here the distinction is that 2 ion peaks are detected in the range of mass 500 to 650. The CI mass spectrum of peak 19 is shown in Fig. 9. The 2 different fatty acid molecules will be referred to as fatty acid 1, 2, and 3. The molecular weights of the two kinds of fatty acids of triglyceride will be referred to a fatty acid molecular weight A and B.

Molecular weight calculation for peak 19: 796 - 18 = 778
Calculation for peak 19 fatty acid molecular weight A: 778 - 523 + 1 = 256
Calculation for peak 19 fatty acid molecular weight B: 778 - 551 + 1 = 228

As with the other pattern, the triglyceride fatty acid molecular weights are presumed to be 256 and 228. Next, it is necessary to determine which fatty acid includes 2 molecules and which is 1 molecule. Usually one of the ions in the EI mass spectrum will be have a significantly higher intensity than the other.

In the EI mass spectrum of peak 19, the intensity of mass 523 is significantly higher than the intensity of mass 551. The ion with the higher intensity usually corresponds to the fatty acid that is attached at two positions in the triglyceride. Therefore, in the case of this compound, the fatty acid with molecular weight 256 corresponding to fatty acid molecular weight A is the one with 2 molecules, and the fatty acid with a molecular weight of 228 is presumed to be the one with 1 molecule. In some analyses, there will not be a significant difference in the intensity of the two ions. In these cases, the method of reconciling the molecular weight determined from the CI result with the calculated fatty acid molecular weight from the two possible structures is effective for determining the correct structure.

For instance, if the molecular weight is calculated on the assumption that one molecule of the fatty acid having a molecular weight of 256 is present, and there are two molecules of the fatty acid of molecular weight 228 present, there will be a discrepancy between [256 + 228 + 228 + 38 = 750] and the expected molecular weight of 778 from the CI results.

On the other hand, assuming that two fatty acids of molecular weight 256 are present and one molecule of the fatty acid of molecular weight 228 is present, the calculation [256 + 256 + 228 + 38 = 778] yields a result which matches the expected molecular weight of peak 19 from the CI result.

The fatty acid of molecular weight 256 is presumed to comprise 2 molecules and the fatty acid of molecular weight 228, one molecule, according to the calculation.

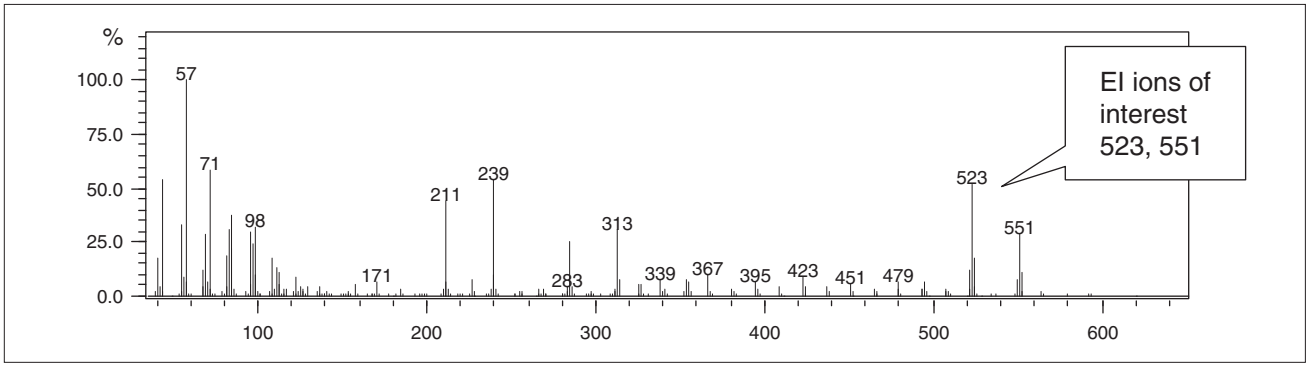

Fig.8 EI Mass Spectrum of Peak #19

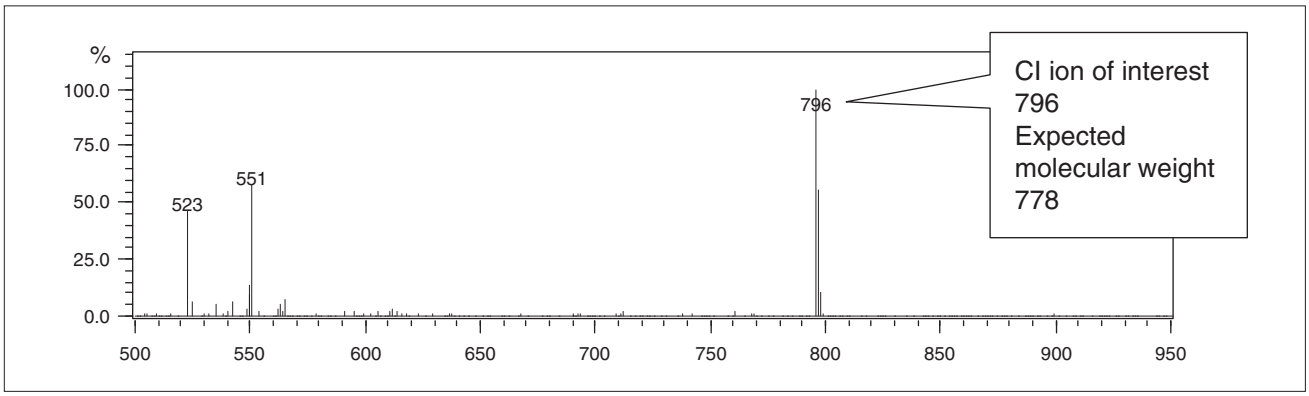

Fig.9 CI Mass Spectrum of Peak #19

NOTES:
✻This Application News has been produced and edited using information that was available when the data was acquired for each article. This Application News is subject to revision without prior notice.

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