Study on triacylglycerides in edible vegetable oils by ultra-high performance liquid chromatography tandem quadrupole-time of flight mass spectrometry

Introduction

Edible vegetable oils constitute an important class of food products, widely used throughout the world. For the food chemist, the determination of the triglyceride (TAG) composition in edible oils provides information that complements that of the fatty acid composition. Knowledge of the distribution of the fatty acids within the glyceride molecules is used for nutritional purposes.

In principle, either GC or HPLC can be used to determine the triglyceride profile of fats and oils. GC analysis using robust columns with high temperature limits can quickly provide the carbon numbers of the triglycerides. However, HPLC analysis provides better resolution between triglycerides of the same carbon number. GC methods have problems with the complexity of sample preprocessing, which requires saponification. Triglycerides are known to be difficult compounds to analyze by HPLC using traditional detectors as the applicability of UV detectors for triglyceride analysis is limited. If the purpose of the analysis is to gain a better knowledge of the fatty acid composition of each triglyceride, LC coupled with MS detection is recommended. In this study, UHPLC-HRMS with positive mode electrospray ionization (ESI+) were used to realize the analysis and identification of triglyceride in edible vegetable oils.

Experimental

Sample preparation

Edible vegetable oils were purchased at local supermarkets. 100mg (±0.1mg) of oil was transferred to a 10mL volumetric and dissolved with 10mL of isopropanol (IPA). The dilutions were vigorously shaken, and 1mL of extract was transferred into an autosampler vial for UHPLC/HRMS analysis.

Instrumentation

UHPLC-QTOF system consists of the Agilent 1290 Infinity II, binary pump, autosampler, TCC, and 6545 LC/Q-TOF with Agilent Jet Stream. The UHPLC-Q-TOF experimental conditions are summarized in Table 1. The chemical formulae were calculated based on accuracy mass and isotope ratio calculation by MassHunter Qualitative Analysis software.

<table>
<thead>
<tr>
<th>Table 1 UHPLC-QTOF Conditions</th>
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</thead>
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<tr>
<td><strong>Column</strong></td>
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| **Mobile phase** | A=2mM acetonitrile containing 10% 10mM ammonium formate and 0.2% formic acid  
B=Isopropanol containing 2% water and 0.2% formic acid |
| **Flow rate** | 0.3mL/min |
| **Column temperature** | 40°C |
| **Injection volume** | 1μL |
| **Gradient Program** | TIME(min) | B(%) |
| | 0.00 | 25 |
| | 3.00 | 25 |
| | 20.0 | 80 |
| | 23.0 | 80 |
| | 23.1 | 25 |
| **Post Time** | 3 min |
| **AJS Dual ESI source** | Ion mode: positive  
-Capillary voltage: 4000V  
-Nozzle voltage: 500V  
-Drying gas: 7L/min@300°C  
-Sheath gas: 11L/min@350°C  
-Nebulizer: 35psi  
-Fragmentor: 170V  
-MS scan: 100-1500 m/z |
Results and Discussion

Intact triglycerides generally have very low water solubility and as such are commonly separated by normal phase chromatography, which separates species largely based on differences in polar functional groups. This chromatography approach has challenges when trying to apply it to routine analysis for triglycerides in edible vegetable oils. Reverse phase chromatography operating in a non-aqueous mode of separation, which has more selectivity for small differences on carbon character such as chain length or degree of saturation, and is easier to use for routine analysis. A typical gradient separation TIC of triglycerides from peanut oil using acetonitrile/IPA gradient is shown in Figure 1.

Most of the LC-MS methods used atmospheric pressure chemical ionization(APCI) as ionization mode for triglycerides. However, its stability is lower than that of ESI ionization mode, due to the problem of corona needle carbonization. By adding a small amount of water and ammonium formate in the mobile phase, formation of [M+NH₄]⁺ ammonium adduct ions was promoted, which are more suitable for ESI analysis.

Through the optimization of chromatographic separation and LC/MS source conditions for ionization, 28 kinds of triglyceride in peanut oil were identified. EICs of triglycerides from peanut oil is shown in Figure 2. The 8 groups (I-VIII) of triglyceride clusters are clearly separated from each other. Each group contains triglycerides similar to carbon chain length or unsaturation. The vast majority of triglycerides ionize the peak of [M+NH₄]⁺, but also contain [M+Na⁺] mass spectrum peak. Typical spectral pattern (from group II) is shown in Figure 3.

Triglycerides in plants are made up predominantly of fatty acids with even numbers of carbons (C₁₄, C₁₆, C₁₈, etc.) and either 0, 1, or 2 double bonds per acid. The main fatty acids include: M, myristic acid (14:0); P, palmitic acid (16:0); S, stearic acid (18:0); O, oleic acid (18:1); L, linoleic acid (18:2); Ln, linolenic acid (18:3); E, Eicosanoic acid

![Figure 1](image1.png)  
**Figure 1.** TIC of TAGs from peanut oil

![Figure 2](image2.png)  
**Figure 2.** EIC of TAGs from peanut oil

![Figure 3](image3.png)  
**Figure 3.** Mass spectrum of a mixed TAGs from group II

![Figure 4](image4.png)  
**Figure 4.** Fragment mass spectrum of typical TAG (LLP)
Results and Discussion

(20:0); Ee, Eicosenoic acid (20:1); De, Docosenoic acid (22:1); Lg, Lignoceric acid (24:0); N, Nervonic acid (24:1).

The information (types of fatty acids and their positions) is important for nutritional purposes, the identification and characterization of fatty acids of triglycerides can be satisfied by MS/MS mode with collision induced dissociation (CID). The No.2 triglyceride of group II of MS/MS confirmation can be obtained using CID, this is shown in Figure 4. The kinds of fatty acids can be identified by MS/MS fragmentation information of loss of fatty acid. The main ion fragments m/z 575.5026 and m/z 599.5028 respectively correspond to linoleic acid (2) and palmitic acid (1), the fatty acid composition (LLP) of the No.2 peak from group II can be identified by MS and MS/MS information. The 8 groups of triglyceride and fatty acid composition of peanut oil were successfully identified by UHPLC-HRMS with ESI+ mode. Detailed information in Table 2.

Table 2. Composition of TAGs from peanut oil

<table>
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<tr>
<th>Group</th>
<th>No.</th>
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Because of the complex molecular species and isomers in fatty acids, the use of a single liquid phase system will be difficult for the separation of triglycerides in natural oils, as a result of interference caused by superposition, which led to the accuracy of fatty acid identification. For the separation of complex samples, it is very effective to use the two dimensional liquid chromatography (2D-LC). This separation mode have more selectivity for triglycerides on carbon character such as chain length or unsaturation, shown in Figure 5.

Conclusions

-A fast UHPLC-HRMS with positive mode electrospray ionization(ESI+) method with a minimum of sample preparation was used to detect the triglycerides of edible vegetable oils.

-Optimized reverse phase chromatography coupled to high resolution LC/MS/MS provided a highly effective method to characterize the fatty acid composition of nutritional glyceride in edible oil.

-A more detailed analysis of the triglycerides in edible vegetable oils by 2D-LC provided even greater separation of complex families of triglycerides is being pursued in further research.

References

[1] Hiroki Kumagai, Agilent Technologies publication 5988-4235EN.
[3] Doug McIntyre, Agilent Technologies publication 5989-8441EN.
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