Analysis of Triglycerides in Cooking Oils Using MALDI-TOF Mass Spectrometry and Principal Component Analysis

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1. Abstract

Triglycerides are composed of three esterified fatty acids bound to a glycerol backbone, and are the main component of several types of cooking oils. They are biologically important molecules in that they serve as an important source of energy. The analysis of lipids using other forms of mass spectrometry has proved difficult due to the large degree of fragmentation observed in the ionization process, indicating the importance of developing a new method. In this study, the analysis of triglycerides in 9 common cooking oils was conducted using matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry. Using 2,5-dihydroxybenzoic acid (2,5-DHB) and 9-aminoacridine (9-aa) as a matrix, intact triglycerides were successfully identified in all 9e cooking oils with little fragmentation. Principal component analysis (PCA), a technique used to identify underlying similarities and differences in a data set, was used in conjunction with MALDI-TOF mass spectrometry. The application of PCA to the mass spectral data obtained from the MALDI-TOF mass spectrometer allowed for further comparison of the underlying properties and components of each cooking oil. The results from PCA show noticeable differences in particular oils, and the MALDI method was also able to discriminate between these differences. This MALDI method can potentially be used to identify unknown oil samples or provide quality control in commercial settings.

2. Acknowledgements

I would like to thank my supervisor, Dr. Kingsley Donkor for the opportunity to conduct research under his supervision. I would also like to thank Western Diversification Canada for funding the MALDI-TOF instrument as well as the Chemistry Department of Thompson Rivers University for the provision of reagents.

3. Introduction

3.1 Triglycerides

Triglycerides are biologically important molecules that serve as an important source of energy in animals. They are composed of a glycerol backbone with three esterified fatty acids bound to it. [1] The length of each of the bound fatty acid chains and the degrees of unsaturation vary between triglycerides.

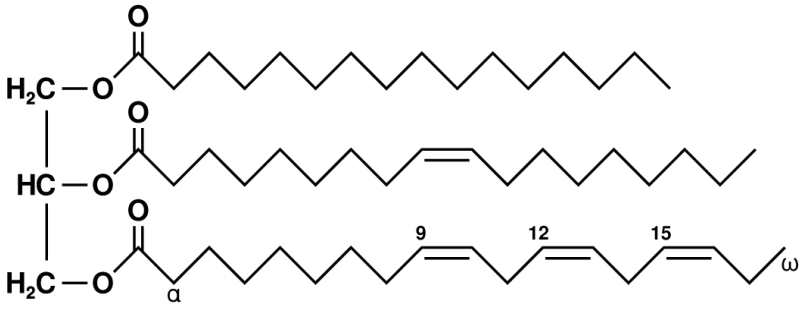

Figure 1: Chemical structure of a general triglyceride

Triglycerides are found in both animal fats and plant oils. Within animal fat, the fatty acid chains are commonly saturated, having no double bonds. Plant triglycerides, however, display a greater degree of unsaturation within their fatty acid chains. Cooking oils are largely made up of a variety of different types of plant triglycerides and have widespread use in commercial settings. With such a large dependence placed on cooking oils, it has become necessary for the development of a method for the fast and reliable analysis of these compounds. Similar to other large biomolecules, however, it is difficult to acquire mass spectral data on triglycerides due to the extensive fragmentation observed during the ionization process. [2]

3.2 MALDI-TOF Mass Spectrometry

Matrix assisted laser desorption/ionization (MALDI) is a soft ionization technique that utilizes a laser to desorb the sample.[3] MALDI is an effective method for the mass spectral analysis of large biomolecules and polymers, which tend to fragment under other forms of mass spectrometry. The sample is first dissolved in a weak acid or a weak base, called a matrix, and pipetted onto a stainless steel plate. The matrix will ionize the sample as well as absorb much of the energy that is introduced by the laser, reducing the amount of fragmentation that occurs. MALDI is commonly coupled with a time-of-flight detector (TOF) that will determine the masses of ions based on the time they take to reach the detector. The desorbed sample is accelerated down the column, where it will reflect off a mirror and strike a detector placed within the column. The detector will then determine the mass of ions based on the time it took for them to reach the detectors.

3.3 Principal Component Analysis

Principal component analysis (PCA) is a chemometric technique used to identify the underlying similarities and differences in a set of data.[4] In a set of correlated data, redundancy is removed via orthogonal transformation. This transformation will yield multiple plots in which the axes are PCs, which are organized in such a way that the first PC will contain the most variation, followed by the second PC, and so on. Additionally, PCA results include a scores plot and a loadings plot. The former plot graphs data based on how similar or different the individuals are to each other, as data with similar properties will be clustered together, while those that are different are farther apart. The loadings plot reveals how each of the variables contribute to a particular PC. Variables with large values on either the x or y axis contribute more to a particular PC than those that have values closer to zero.

4. Methods

4.1 Reagents

Matrices 2,5-dihydroxybenzoic (2,5-DHB) acid and 9-aminoacridine (9-aa) were purchased from Sigma Aldrich. Various solvents used, including hexane, isopropanol, and acetonitrile, were supplied by the TRU Chemistry Department. Eight common cooking oils were purchased and are summarized in Table 1.

Table 1: Cooking oils used and their respective brand names

Oil Sample	Brand Name
Grapeseed	Carapelli
Corn	No Name
Canola	Spectrum Naturals
Olive	Western Family
Sesame	Six Fortune
Peanut	Planters
Vegetable	Crisco
Sunflower	Safflo

4.2 Sample Preparation

Each cooking oil sample was diluted to 0.05% v/v in hexane. Matrices 2,5-DHB and 9-aa were separately dissolved in an isopropanol/acetonitrile (60/40 ratio) solution to a final concentration of 10mg/mL. The sample oils were mixed in a 1:1 ratio with the matrix solutions and 1µL of each sample was spotted onto a stainless steel MALDI plate for MALDI-TOF analysis (Figure 2).

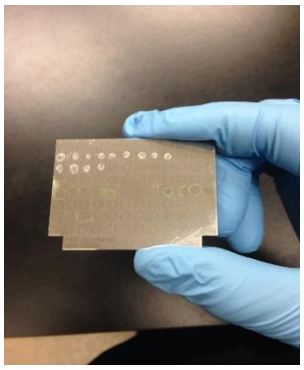

Figure 2: Samples spotted onto stainless steel MALDI plate

4.3 MALDI-TOF Analysis

MALDI-TOF analysis was carried out using a benchtop *Microflex* MALDI-TOF mass spectrometer from Bruker Daltonics (Figure 3). Optimized instrument parameters are summarized in Table 2.

Figure 3: Benchtop *Microflex* MALDI-TOF mass spectrometer from Bruker Daltonics

Table 2: Optimized MALDI-TOF Instrument Parameters

Optimized Parameters	
Laser Type	LTB MNL 100 (106 PD) V 002.61
Laser Wavelength	355 nm
Laser Frequency	60.0 Hz
Initial Laser Power	30%
Shots at Raster Spot	10
Total Number of Shots	300
Linear Detector Voltage	2.50 kV
Ion Source 1	19.00 kV
Ion Source 2	15.68 kV
Lens Voltage	9.44 kV
Reflector Voltage	19.98 kV
Pulsed Ion Extraction	50 ns
Polarity	Positive

The stainless steel MALDI plate was inserted into the MALDI-TOF instrument. Each of the eight oil samples in each matrix was analyzed in triplicate.

4.4 Principal Component Analysis

The mass-to-charge ratio and intensity obtained from the mass spectra from the MALDI-TOF analysis for each oil was then copied into an Excel table. Values that did not correlate with anything were removed until a correlated data set was produced. This Excel table was then

exported to a software program called The Unscrambler 9.1 (provided by TRU), which performed PCA on the two sets of data with the 2,5-DHB matrix and the 9-aa matrix.

5. Results and Discussion

5.1 Mass Spectra

Three mass spectra were obtained for each oil, for a total of 24 spectra for each matrix. The samples were run in triplicate to ensure that there was consistency among the data that was gathered from the MALDI-TOF analysis. The best spectra of the three, those with minimal background noise were then selected for PCA. Spectra obtained from the sunflower oil, canola oil, and grapeseed oil samples in each matrix are shown in Figure 4.

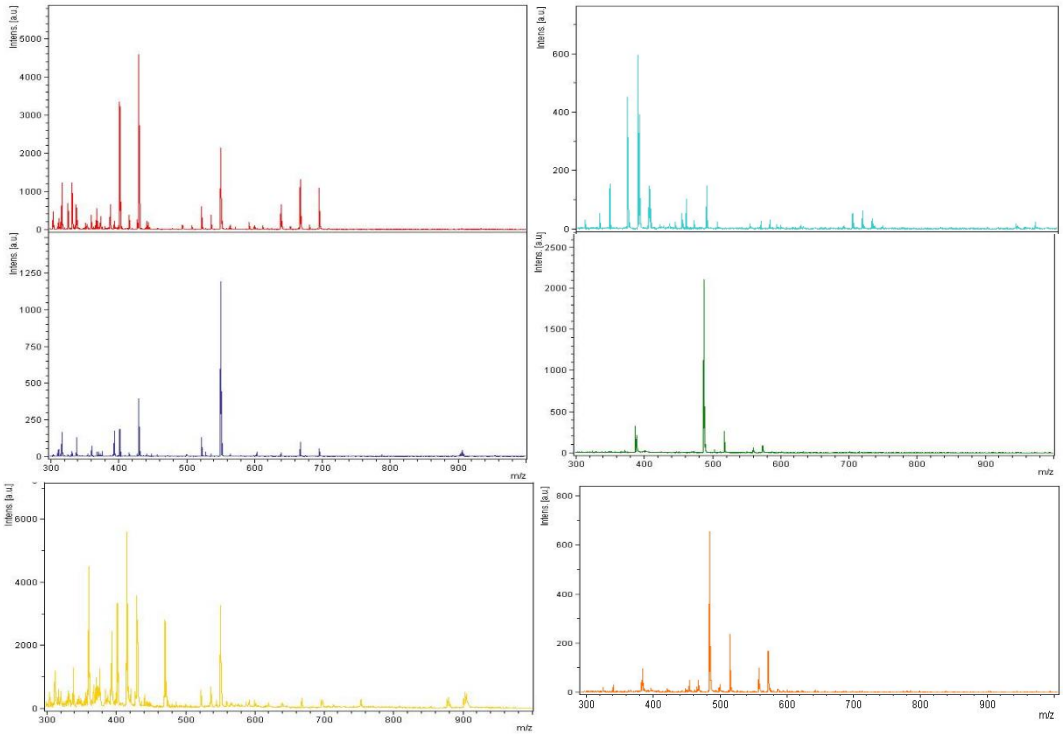

Figure 4: MALDI-TOF mass spectra of grapeseed (top), canola (middle), and sunflower (bottom) oil samples in 2,5-DHB (left) and 9-aa (right)

Triglycerides were identified in both matrices, however there was some fragmentation observed in each oil sample. In the oil samples mixed with 2,5-DHB, extensive fragmentation was observed, suggesting that this matrix is not suitable for the analysis for triglycerides. In contrast, the oil samples in the 9-aa matrix displayed a much smaller degree of fragmentation, as there were far fewer peaks in the spectra obtained. This was a strange finding, due to its lack of consistency with the work done by Hidaka and colleagues[5]. Their study on the analysis of

lipoprotein lipid composition in human serum identified intact triglycerides with minimal fragmentation using 2,5-DHB as a matrix. An explanation for these observed differences could be due to the presence of other compounds found within cooking oils, such as diacylglycerols.[6] It is possible that the fragmentation observed was due to the fragmentation of these other compounds as opposed to the triglycerides themselves.

5.2 Principal Component Analysis

A scores plot was obtained from The Unscrambler 9.1 software for each matrix and is shown in Figure 5.

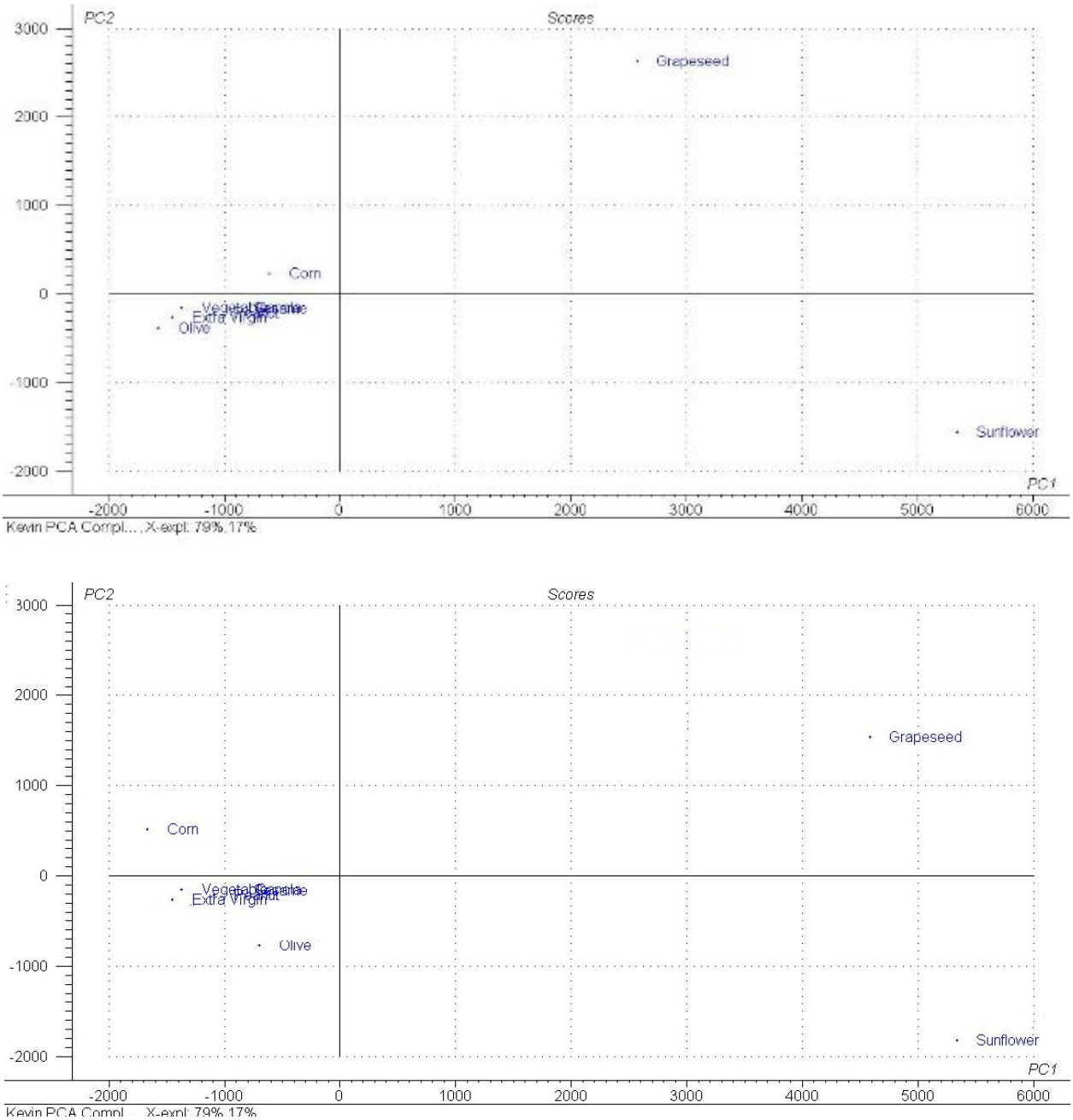

Figure 5: Scores plot obtained from PCA for oil samples in 2,5-DHB (top) and 9-aa (bottom)

The scores plot revealed three general clusters. The first contained corn, olive, vegetable, canola, sesame, and canola oil. The second contained grapeseed oil only, and the third sunflower oil only. The scores plot obtained from the mass spectral data from the 2,5-DHB matrix was very similar to the scores plot obtained from the 9-aa matrix, suggesting that the data obtained were very consistent. Comparing these sets of data to Figure 4, there are also some consistencies observed. The two scores plots showed three clusters that have different properties from one

another. This was also reflected in the mass spectra obtained from the canola oil (a representative of the first cluster on the scores plot), the grapeseed oil, and the sunflower oil. This suggests that MALDI-TOF is sensitive to small differences in the compositions of different cooking oils. These findings are also consistent with the literature.[7] Corn, canola, olive, peanut, vegetable, and sesame oil are all largely composed of oleic acid. The presence of oleic acid in all of these oils could explain why they are clustered so closely together in the scores plot in Figure 5. Additionally, the scores plot for each matrix show these six oils clustered together and the mass spectra obtained have similar peaks and intensities. While sunflower oil and grapeseed oil both contain linoleic acid, they differ in the other types of triglycerides that make up the oil. This may explain why these two oils are found so far apart on the scores plots and why each have different mass spectra. Additionally, they may also differ in the composition of diacylglycerols and other compounds found within the cooking oils.

These results show that, while fragmentation was observed in both of the matrices, the MALDI-TOF method developed was sensitive to small differences in the lipid compositions of each cooking oil, as confirmed by the PCA data and the literature. Consequently, this method could potentially be applied to a commercial level, where it may be used as a form of quality control in the cooking industry.

6. Conclusion

The MALDI-TOF mass spectrometry method successfully identified triglycerides in each of the cooking oils. Although there was fragmentation observed in both of the matrices, it appeared that the 9-aa matrix was more suitable for the analysis of triglycerides in these cooking oils, as it displayed a much smaller degree of fragmentation. Finally, MALDI was able to discriminate between small differences in cooking oil composition, as confirmed by the PCA data and literature findings, making this a possibly suitable method for triglyceride analysis in the cooking oil industry as a form of quality control.

7. Literature Cited

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