



ABSTRACT OF THE DOCTORAL THESIS

CHARACTERIZATION OF PLANT OILS BASED ON THEIR TRIACYLGLYCEROL CONTENT BY HPLC/APCI-MS AND MALDI-TOFMS

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I. INTRODUCTION, AIM

Edible oils play an important role in human nutrition due to their everyday consumption and biologically important compounds present in them. The composition of the different types of oils is considerably diverse, but in general they contain significant amount of triacylglycerols (*ca.* 97%) and biologically also important minor compounds. Despite the large number of publications describing the importance of the oils in nutrition, biochemistry and also many other areas of science, only few are focused on their analysis. Their analysis by modern analytical techniques such as high-performance liquid chromatography coupled to mass spectrometry (HPLC/MS) has become straightforward after the development of atmospheric pressure chemical ionization (APCI). This is due to that APCI yields simple mass spectra from triacylglycerols and provides also the possibility of distinguish between the positional isomers. During the fragmentation, fatty acid eliminates from the molecule (Figure 1) and the probability of fatty acid loss depends on its position.

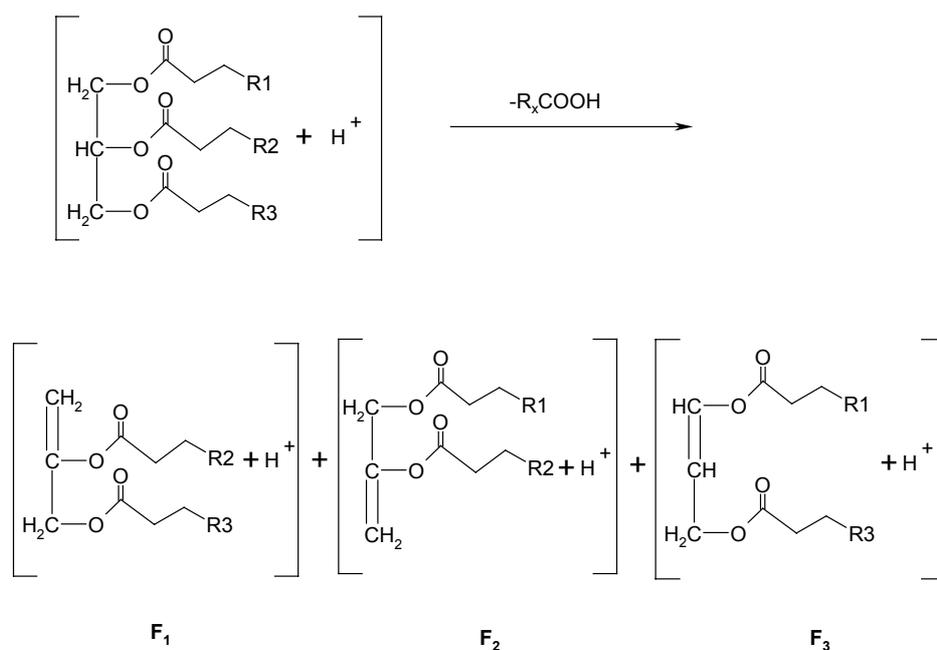


Figure 1. The most dominant APCI fragmentation pathway of triacylglycerols. (The sketched objects depict possible ion structures, but not necessary represent the real ones.)

Although the analysis of plant oils -mainly their triacylglycerol compounds- by HPLC/MS has become more popular than analysis by modern analytical techniques such as HPLC/APCI-MS and matrix assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOFMS) is still not significant.

The doctoral thesis attempted to perform a comprehensive examination of various types of oils focusing on their triacylglycerol composition by HPLC/APCI-MS and MALDI-TOFMS, and also in combination with statistical analysis.

II. EXPERIMENTAL

Analyses of plant oil triacylglycerol profiles were performed by using two different mass spectrometric methods, **HPLC/APCI-MS** and **MALDI-TOFMS**. HPLC/APCI-MS measurements were performed on a Shimadzu QP2010 mass spectrometer coupled to a Shimadzu HPLC system. MALDI-TOFMS measurements were performed on a Bruker BIFLEX mass spectrometer in reflectron mode. To evaluate the triacylglycerol profiles of different types of plant oils **linear discriminant analysis** (LDA) was performed using STATISTICA software package. Two different reversed-phase HPLC columns were applied during the HPLC/APCI-MS measurements, (i) a **conventional microparticulate** (Purospher, RP-18e, 125x4 mm, 5 µm, Merck) and (ii) a **monolithic** (SilicaROD, RP-18e, 50x4.6 mm, Merck) **column**.

Quantitation of the ratio of **dilinoleoyl-oleoyl glycerol positional isomers** (LLO and LOL) in plant oils was performed by HPLC/APCI-MS in **selected ion monitoring** (SIM) mode. The structures of the LLO and LOL isomer standards were confirmed by APCI-MS and nuclear magnetic resonance (NMR) analyses.

III. NEW RESULTS AND CONCLUSIONS

- III.1. Different methods were worked out for characterization of plant oils based on their triacylglycerol profile by HPLC/APCI-MS and MALDI-TOFMS in combination with linear discriminant analysis.
- III.2. Successful classification of 14 different types of plant oils (almond, avocado, corn germ, grape seed, linseed, mustard seed, olive, peanut, pumpkin seed, sesame seed, soybean, sunflower, walnut and wheat germ) was obtained by linear discriminant analysis based on their HPLC/APCI-MS triacylglycerol profiles. Out of the 73 samples 68 were correctly classified (93.2%) indicating correct classification of 9 different types of oils (almond, avocado, grape seed, linseed, mustard seed, olive, pumpkin seed, sesame seed and soybean).
- III.3. Successful classification of the 14 different types of plant oils was obtained also by linear discriminant analysis based on their MALDI-TOFMS triacylglycerol profiles. Out of the 73 samples 68 were correctly classified (93.2%) in this case also, indicating correct classification of 12 different types of oils (almond, avocado, corn germ, grape seed, linseed, mustard seed, olive, peanut, sesame seed, soybean, sunflower and walnut). Comparing the two mass spectrometric methods combined with LDA, MALDI-TOFMS provided better results in addition of better repeatability of the measurements, much shorter analysis and data processing time.

- III.4. Adequate and repeatable separation of plant oil triacylglycerols was achieved within a very short analysis time (10 min total run time) on a monolithic RP silica column (SilicaROD, RP-18e, 50x4.6 mm, Merck) using gradient elution with acetone-acetonitrile. Standard deviation of the retention times was 0.8% indicating the good repeatability of the measurements.
- III.5. The short-run HPLC method (III.4.) was successfully coupled to APCI mass spectrometer by splitting the eluent flow resulting *ca.* 400 $\mu\text{L}\cdot\text{min}^{-1}$. The standard deviations of the peak areas were around 10% (12% and 7% at small and large peaks, respectively).
- III.6. LOL standard was synthesized in order to quantitate the LLO and LOL isomer ratio based on their diacylglycerol fragments ($[\text{LL}]^+$, $[\text{LO}]^+$). The structure difference between the LLO and LOL standards was confirmed by HPLC/APCI-MS and NMR. The calibration curve of the diacylglycerol fragment ratio ($[\text{LL}]^+ / [\text{LO}]^+$, %) in various LOL (and LLO) concentrations was measured in SIM mode. This was found to be linear ($r=0,9942$, $r_{\text{krit}}=0,6020$) making possible the determination of the exact ratio of the LOL and LLO isomers in various oils.
- III.7. The relative LOL content were measured in grape seed, sunflower, olive, pumpkin seed, soybean and wheat germ by HPLC/APCI-MS in SIM mode and was found to be a constant value per oil varieties. The relative LOL contents in increasing order were accounted for 0%, 13.9 \pm 4.3%, 15.9 \pm 2.9%, 16.7 \pm 4.6%, 26.8 \pm 3.2% and 44.2 \pm 2.6% in olive, wheat germ, soybean, pumpkin seed, sunflower and grape seed oils, respectively. Olive oils contained practically

100% of LLO isomer confirming the published data. This constant ratio of the LOL and LLO isomers per oil varieties indicates that the unsaturated fatty acids such as linoleic and oleic acids have “non-random” distribution pattern in various oils.

IV. USEFULNESS OF THE RESULTS

Classification and authentication of different plant oils can be performed by the methods described in the thesis.

Evaluation of the effect of the LOL and LLO positional isomer triacylglycerols on human nutrition and metabolism can also be investigated in indirect ways with the knowledge of the exact ratio of these positional isomers in grape seed, sunflower, olive, pumpkin seed, soybean and wheat germ oils.

V. PUBLICATIONS (related to the thesis)

Published articles:

1. A. Jakab, K. Nagy, K. Héberger, K. Vékey, E. Forgács, Differentiation of Vegetable Oils by Mass Spectrometry Combined with Statistical Analysis, *Rapid Commun. Mass Spectrom.* 2002; **16/24**: 2291-2297.
2. A. Jakab, K. Héberger, E. Forgács, Comparative Analysis of Different Plant Oils by High-Performance Liquid Chromatography-Atmospheric Pressure Chemical Ionization Mass Spectrometry, *J. Chromatogr. A*, 2002; **976**: 255-263.
3. A. Jakab, E. Forgács, Characterization of Plant Oils on a Monolithic Silica Column by High-Performance Liquid Chromatography-Atmospheric Pressure Chemical Ionization Mass Spectrometry, *Chromatographia*, 2002; **56**: S69-S73.

Submitted article:

4. A. Jakab, I. Jablonkai, E. Forgács, Quantitation of positional isomer dilinoleoyl-oleoyl-glycerols in Plant oils by Mass Spectrometry.

Poster presentations (english):

5. A. Jakab, I. Jablonkai, E. Forgács, Quantification of positional isomer triacylglycerols in plant oils, *21th Informal Meeting on Mass Spectrometry* (IMMS 21th), Antwerp, Belgium, 11-15. May 2003.

6. A. Jakab, K. Nagy, K. Vékey, E. Forgács, Analysis of Plant Oil Triacylglycerols by HPLC/APCI-MS and MALDI-TOFMS, *20th Informal Meeting on Mass Spectrometry* (IMMS 20th), Primiero, Italy, 12-16. May 2002.
7. A. Jakab, E. Forgács, Comparative Analysis of Different Plant Oils by HPLC/APCI-MS, *Seventh International Symposium on Hyphenated Techniques in Chromatography and Hyphenated Chromatographic Analyzers* (HTC-7) Bruges, Belgium, 6-8. Feb. 2002.
8. A. Jakab, E. Forgács, Characterization of Plant Oils on a Monolithic Silica Column by HPLC/APCI-MS, *Balaton Symposium '01*, Siófok, Hungary, 2-4. Sept. 2001.

Poster presentation (hungarian):

9. Jakab A., Nagy K., Héberger K., Vékey K., Forgács E., Növényi olajok csoportosítása lineáris diszkriminancia analízissel tömegspektrometriás adatok alapján, *Chemometrics 2002*, Tata, Hungary, 29. Sept-1. Oct. 2002.
10. Jakab A., Forgács E., Növényi olajok természetes antioxidáns tartalmának vizsgálata HPLC/MS-es, *Elválasztástudományi Vándorgyűlés*, Lillafüred, Hungary, 16-18. Oct. 2002.

Oral presentations (hungarian):

11. Jakab A., Nagy K., Vékey K., Forgács E., Növényi Olajok Vizsgálata HPLC/APCI-MS és MALDI-TOFMS módszerekkel, *V. Doktori Kémiai Iskola*, Szokolya-Királyrét, 2002. május 21-22.

12. Jakab A., Forgács E., Növényi Olajok HPLC/APCI-MS Vizsgálata, **307. Tudományos Kollokvium**, FVM Központi Élelmiszeripari Kutató Intézet, Budapest, 2002. február 22.