High-Throughput Cyclolinopeptide and Triacylglycerol (TAG) Profiling of *Linum usitatissimum* using LDTD-MS/MS

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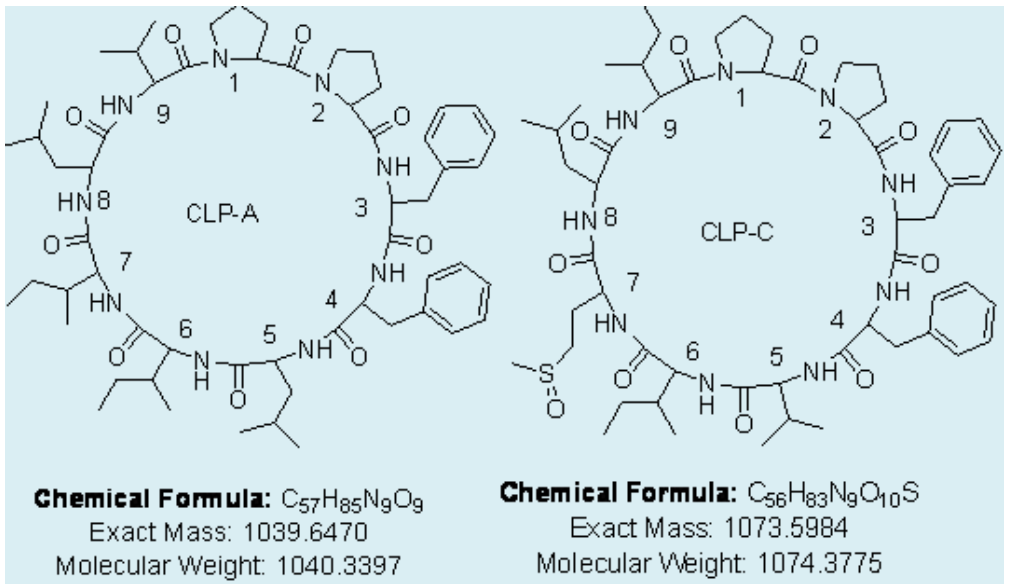
Introduction

Plant material, such as flax seeds and flax oil contain cyclolinopeptides (CLP) and triacylglycerol (TAG) compounds. The biological activity of some cyclolinopeptides has been reported to include immunosuppressive activity. Therefore, it is important to identify the cyclolinopeptides contained in the plant extract. TAGs are the major part of naturally occurring fats and oils in plant and the TAG composition and structure define the physiological effects of fats and oils in human diet. Moreover, TAGs are also used as inexpensive feedstock in the manufacture of biodiesel. The ability to characterize and conduct high throughput profiling of CLPs and TAGs in flax is very useful. LDTD-MS/MS was used to characterize these compounds in flax seed extracts. An example of two common cyclolinopeptides are shown below.



Chemical Formula: $C_{57}H_{85}N_9O_9$
Exact Mass: 1039.6470
Molecular Weight: 1040.3397

Chemical Formula: $C_{56}H_{83}N_9O_{10}S$
Exact Mass: 1073.5984
Molecular Weight: 1074.3775

Experimental

Sample Preparation

Flax oil was obtained from Bioriginal Foods & Science Corp (Saskatoon, SK). The flax seed was from the Crop Development Centre (Saskatoon, SK). Different techniques were used to extract cyclolinopeptides (CLPs) in oil and seed. Sample preparation was performed by passing flax oil (2 mL) through a silica gel column or mixing ground flax seed (1 gram) with silica gel then washing with hexane, followed by 1:1 ethyl acetate:hexane. The CLP/TAGS fraction was obtained by eluting with methanol/dichloromethane (1:9). A second sample of oil (2 mL) was mixed with methanol (1:1) and extracted. The three samples were evaporated to dryness.

Experimental

Sample Analysis

The CLP and TAG profiles were analyzed after dissolving the extracts in methanol or chloroform/methanol (1:2), respectively. For the CLPs, 400 µL of methanol was added to the sample vial and vortexed for 1 minute. A 2 µL aliquot was pipetted into the LazWell plate. The solvent was evaporated at room temperature prior to analysis.

The samples were analyzed by LDTD-MS/MS using a Agilent 6410 triple quadrupole mass spectrometer in positive APCI mode with an analysis time of 6 seconds per sample. Each sample was run in triplicate for a total run time of <1 minute each. The samples were run in full scan mode, followed by targeted MS/MS of the main components. The gas flow setting was 3 L/min, fragmentor voltage: 135 V, and collision energy: 40-45 eV.

Phytronix Laser Diode Thermal Desorption (LDTD) ion source

Agilent 6410 QQQ Mass Spectrometer

Results and Discussion

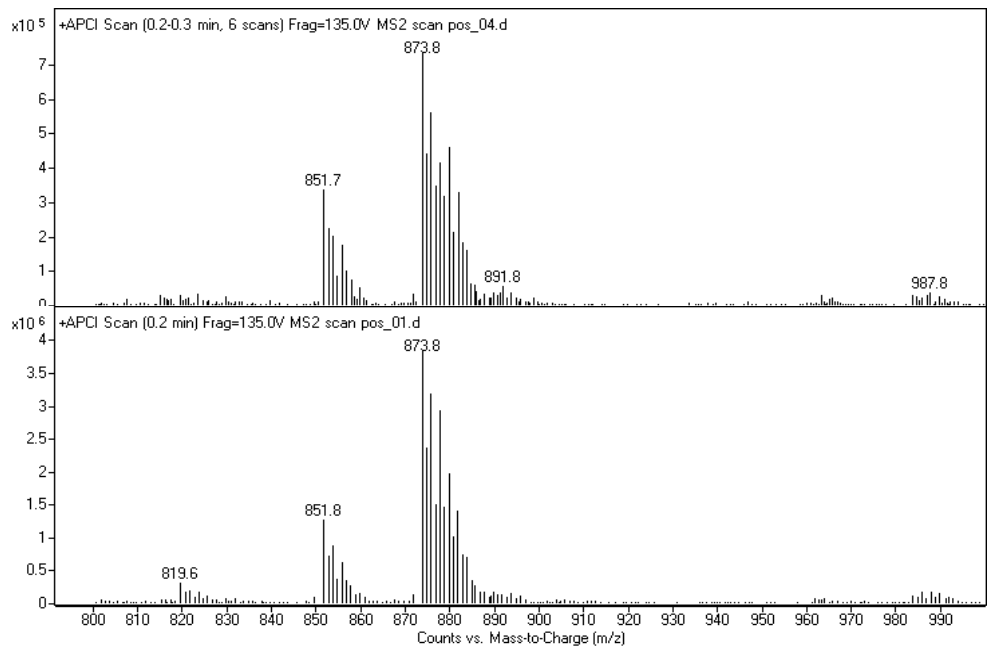


Figure 1: Analysis of TAGs in a) methanol extract and b) silica gel treated flax oil. Full scan spectra were obtained using LDTD-MS in positive APCI mode.

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Results and Discussion

Triacylglycerols (TAGs)

The flax oil samples were analyzed and the major TAGs were selected for MS/MS confirmation. Dissolving the sample with methanol/DCM aided in the extraction of the TAGs. Figure 1 compares the full scan mass spectra of the methanol extracted flax oil with flax oil silica gel *treated. TAG profiling using full scan MS: The main TAG present in the extracts is the LnLnLn at $[M+H]^+$ = 873.7. Other TAGs that were detected included: LnLnP (m/z = 851.7), LnLnO (m/z = 877.7), LnLnS (m/z = 879.7) and OLLn (m/z = 879.7). Each TAG was confirmed using MS/MS analysis. Figure 2 shows the MS/MS spectra of the 4 principle TAGs.*

There were more TAGs extracted from the silica gel preparation. In general, the concentration was four times greater in the silica treated oil. These results were expected due to the greater solubility of TAGs in methanol/DCM (in comparison to methanol only) during the extraction step. *The relative amounts and proposed identities for these compounds is shown in Table 1.*

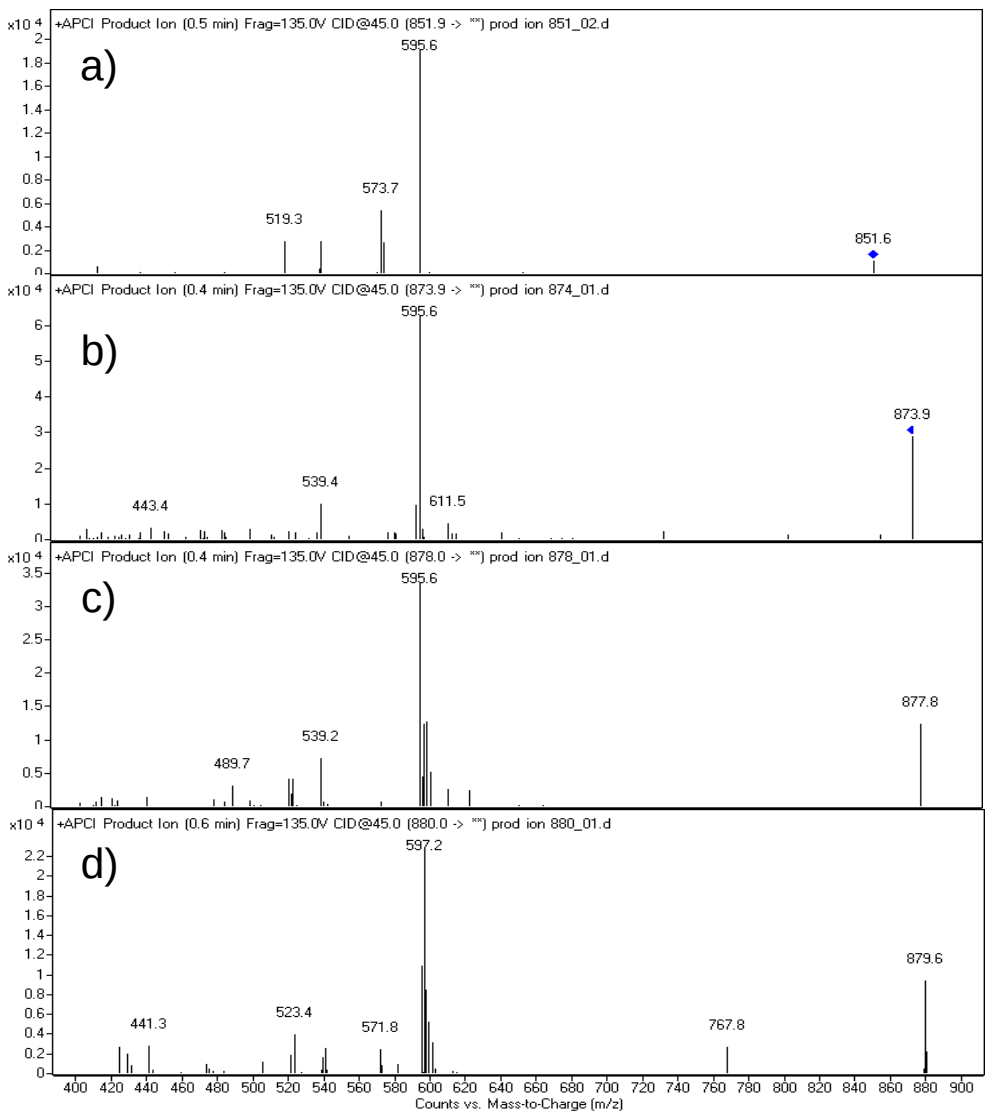

Figure 2: MS/MS Analysis of the main TAGs in flax oil. They have been tentatively identified as a) LnLnP; b) LnLnLn; c) LnLnO; and d) OLLn/LnLnS.

Table 1: Comparison of the relative amounts of selected TAGs in the flax oil after different extraction procedures.

Proposed TAG	*m/z*	*Methanol Extract*	*Silica Treated*
LnLnP	*851.7*	*0.21*	*0.79*
LnLnLn	*873.8*	*0.16*	*0.84*
LnLnO	*877.7*	*0.12*	*0.88*
OLLn/LnLnS	*879.9*	*0.19*	*0.81*

Cyclolinopeptides (CLPs)

The three sample extracts (flax oil extracted with methanol or eluted through a silica gel column and ground flax seed silica gel treated) were analyzed and the major CLPs were selected for MS/MS confirmation. The full scan spectra of the three samples are shown in Figure 3. Overall, more CLPs were identified in the flax seed. Since the source of the oil and seeds were different, no other conclusions can be made about extraction efficiency between the oil and flax seed using the different techniques.

Differences were observed in the distribution and abundance of the CLPs between the oil and seed, but additional studies are required to confirm this preliminary result. A comparison between the extraction efficiency of the two procedures for the flax oil is shown in Table 2. For the flax oil samples, there appeared to be more CLPs extracted in the methanol extract suggesting that the methanol extraction may be more efficient for removal of CLPs from flax oil.

CLP profiling using full scan MS: Five unique CLPs were detected in the different extracts, CLP-A (m/z = 1040.7), CLP-C (m/z = 1074.7), CPL-E (m/z = 977.5), CPL-J (m/z = 993.5), and CPL-K (m/z = 1090.7). All these CLPs have been reported to be present in flax oil and seed, with CLP-A ($C_{57}H_{85}N_9O_9$) being the most predominant. The sequences of these peptides were confirmed based upon their product ion scan.

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The MS/MS spectra and sequence for CLP-A is shown in Figure 4. A novel compound at m/z 1010.6 was detected. However, after additional work, it was determined to be CLP-C-CH_3SOH (-m/z 64). No optimization was conducted for the source conditions and this loss is likely due to excessive source temperature or fragmentor voltage for the more fragile CLPs. The MS/MS spectra and sequence for CLP-C–m/z 64 is shown in Figure 5.

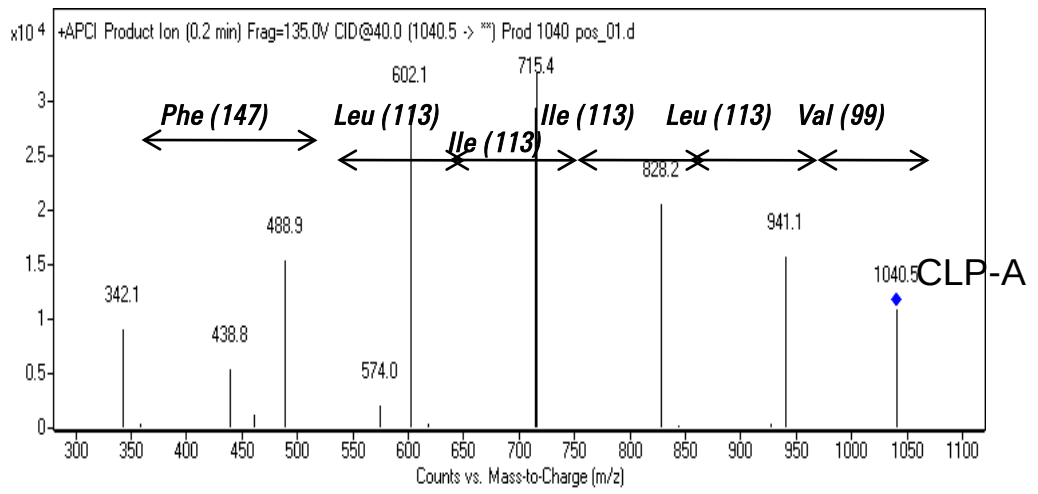

Figure 4: MS/MS spectra and sequence information for CLP-A.

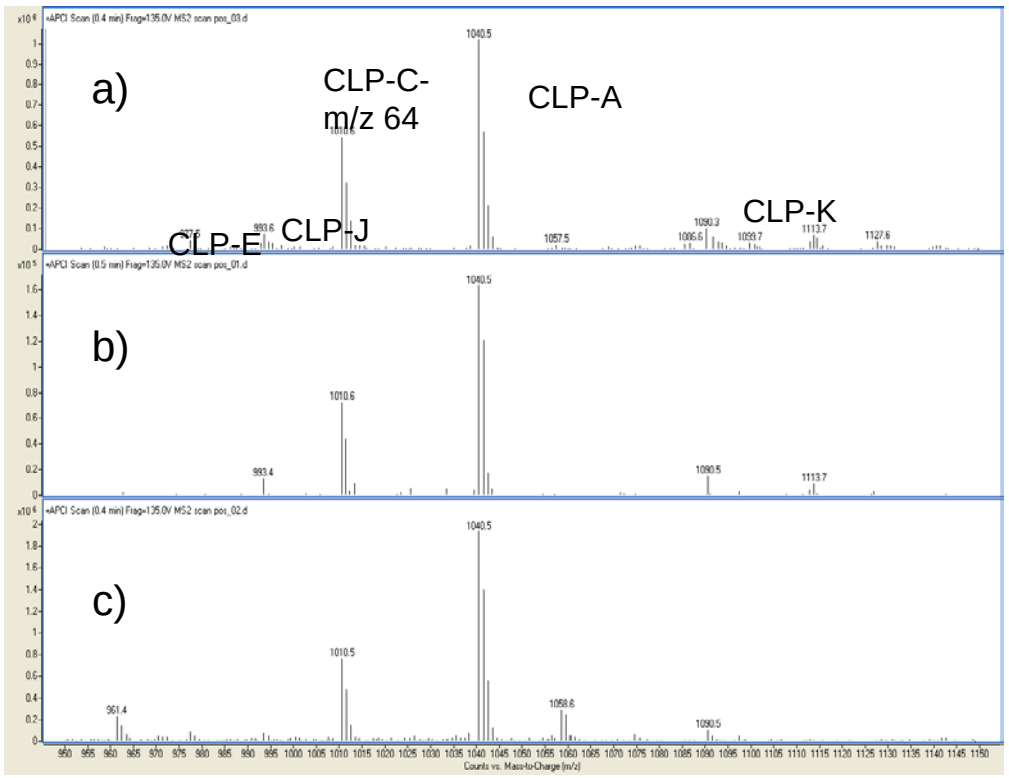

Figure 3: Analysis of the main CLPs found in a) methanol extracted flax oil; b) silica gel treated flax oil and c) silica gel treated flax seed. Full scan spectra were obtained using LDTD-MS in positive APCI mode.

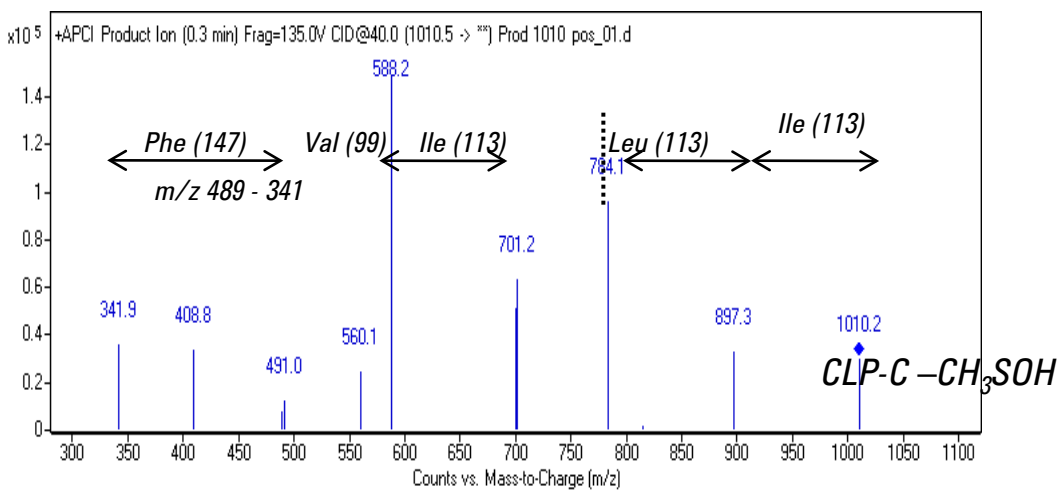

Figure 5: MS/MS spectra and sequence information for CLP-C.

Table 2: Comparison of the relative amounts of selected CLPs in the flax oil after different extraction procedures.

Proposed CLP	*m/z*	*Methanol Extract*	*Silica Treated*
CLP E	*977.5*	*1*	*0*
CLP J	*993.6*	*0.85*	*0.15*
CLP C-m/z 64	*1010.6*	*0.88*	*0.12*
CLP A	*1040.5*	*0.86*	*0.14*
CLP K	*1090.3*	*0.87*	*0.13*

Conclusions

The relative distribution of these CLPs in the various extracts of flax seed and flax oil have the potential to provide useful information on the immunosuppressive activity of flax seeds from different locations and strains. Using LDTD-QQQ, it is possible to get useful quantitative information on this distribution. Another advantage of the technique employed is that it allows rapid screening of a large number of samples.