Characterization of triacylglycerols in milk at the double bond positional level using oxygen attachment dissociation (OAD)

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Overview

- Oxygen Attachment Dissociation (OAD) is a radical-induced dissociation technique which specifically fragments carbon-carbon double bonds and can be used to determine the positions of double bonds (C=C) in complex lipids.
- Simultaneous acquisition of CID-MS/MS and OAD-MS/MS fragments (OAciD) was applied to the analysis of triacylglycerols (TGs) in milk samples to annotate TGs to the fatty acid composition level and generate unique OAD fragment ions to determine the double bond position.
- High resolution accurate mass LC-MS/MS using OAciD enabled the identification of closely eluting TGs which was not possible using only CID.

1. Introduction

OAD is a radical-induced dissociation technique which specifically fragments C=C double bonds and can be used to determine the positions of double bonds in complex lipids. OAD-MS/MS and conventional CID-MS/MS can be applied simultaneously in positive or negative ion mode data acquisition on a high resolution QTOF instrument to generate OAD- and CID-specific fragment ions. This approach was used to characterize C=C positional information in profiling triacylglycerols (TGs) in bovine and buffalo milk.

2. Materials and Methods

Milk samples from bovine and buffalo were prepared by the Folch method (200 µL sample + 50 µL water + 1000 µL solvent - chloroform:methanol 2:1 v/v). The bottom lipid-containing phase was transferred to a glass vial and placed into a speed vac and evaporated to dryness. Samples were reconstituted in 1 mL butanol:methanol:chloroform (3:5:4), then diluted 1:50. Analysis was performed using high resolution LC-MS/MS analysis (QTOF LCMS-9050, Shimadzu Corporation) operating with a single time-of-flight (TOF) MS scan (m/z 100−1000) followed by data dependent acquisition MS/MS mass scans in positive ion mode with optimal conditions for simultaneous acquisition of CID and OAD-MS/MS fragments (OAciD) at 6-30V.

Reversed phase LC Separation.

- Column: Acquity CSH C18 (2.1 x 100 mm1.7 µm); column temp. 50 ºC, flow rate: 0.4 mL/min, 44 min total analysis time.
- A: water + 10mM ammonium formate + 0.1% formic acid.
- B: methanol:2-propanol 85:15 + 10mM ammonium formate + 0.1% formic acid.
- C: 2-propanol.

LC-MS/MS Mass Spectrometry Detection.

- LCMS-9050 equipped with OAD Radical Source I (Shimadzu Corporation, Japan).
- MS scan m/z 100-1000, 50 msec scan time, 0-32 min.
- DDA-MS/MS; up-to 4 dependent scans m/z 100-1000, collision energy spread 6-30V, 300 msec collision gas pressure 17 kPa. Targeted MS/MS was also performed.

Data Processing.

- LabSolutions Insight™ software (Shimadzu Corporation) was used to process data.
- Acquired DDA-MS/MS spectra were analyzed for TG annotation to assign C=C double bond positions and fatty acid composition. Lipids were annotated according to the omega nomenclature, counting carbons from the methyl end of the fatty acyl chain.

3. Results

3.1 Limited annotation of TGs using RT and CID MS/MS

TG annotation by CID-MS/MS is limited to the determination of fatty acid composition by neutral loss fragmentation and can be challenging when multiple TGs are identified in the CID-MS/MS spectrum from a single chromatographic peak, or when (partially) separated chromatographic features contain indistinguishable CID-MS/MS. In the analysis of buffalo and bovine milk extracts, both scenarios were observed. Figure 1 highlights an example, whereby TG 28:1 (observed in lower abundance in bovine milk compared to buffalo), was detected as four partially resolved chromatographic peaks in buffalo, only three of which could be detected in bovine. Amongst the common peaks, CID-MS/MS revealed co-elution of two different TGs at 10.06 min (TG 16:1_8:0_4:0 and TG 14:1_10:0_4:0), while the peak unique to buffalo milk at 10.32 min was annotated as TG 18:1_6:0_4:0 with indistinguishable CID-MS/MS from a peak detected in both milks at 10.02 mins.

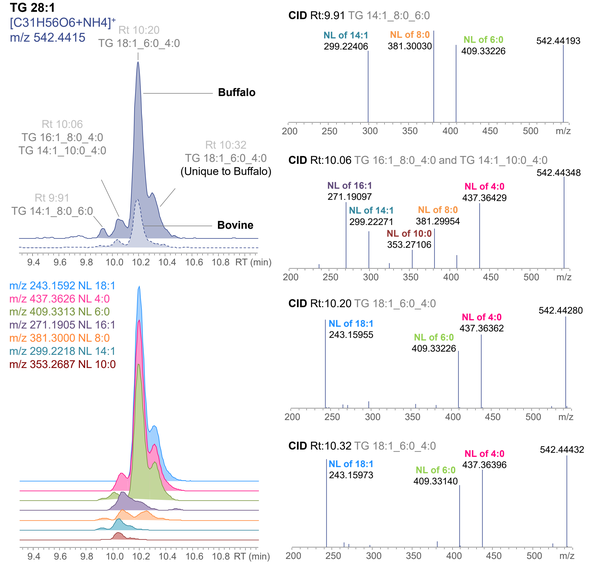

Figure 1. Detection of TG 28:1 isomers in bovine and buffalo milk extracts. Four isomers were detected in buffalo milk the first three of which were common, but at lower abundance, in bovine milk. CID-MS/MS spectra for each of the four labelled peaks and neutral loss (NL) mass chromatograms are presented for the buffalo milk extract, including the fourth peak which was absent in the bovine milk profile.

3.2 Enhanced annotation of TGs using OAciD MS/MS

OAciD (simultaneous acquisition of CID and OAD-MSMS) enables structural characterization of lipids through the determination of C=C double bond positions in the fatty acyl chains. Chromatographically separated lipids with similar CID-MS/MS can be distinguishable with the addition of OAD-MS/MS specific fragment ions. Using the sodium adduct as the precursor ion, the OAciD-MS/MS spectra include specific fragmentation for the $C_{x-1}H_{2x-2}O_{-1}$ ions providing evidence of structurally different fatty acids. Figure 2 highlights the specificity OAciD brings to the annotation of the most abundant isomers of TG 28:1 in buffalo milk extract. The unique TG detected in buffalo could be structurally characterized as TG 18:1(n-7)_6:0_4:0, differentiating it from TG 18:1(n-9)_6:0_4:0 which was present in both milk extracts. Combining the fragment information derived from OAciD on the chimeric MS/MS spectrum for the peak at 10.06 min, present in both bovine and buffalo milk extracts, it was possible to identify the presence of TGs structurally characterized as TG 16:1(n-7)_8:0_4:0 and TG 14:1(n-5)_10:0_4:0.

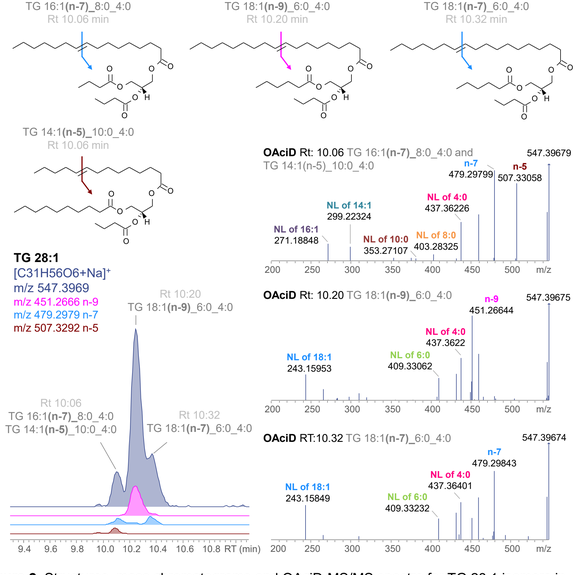

Figure 2. Structures, mass chromatograms and OAciD-MS/MS spectra for TG 28:1 isomers in buffalo milk. OAciD-MS/MS on the sodium adduct precursors generated both CID- and OAD-specific fragment ions enabling the annotation of TG 16:1(n-7)_8:0_4:0, TG 14:1(n-5)_10:0_4:0, TG 18:1(n-9)_6:0_4:0 and TG 18:1(n-7)_6:0_4:0. Spectral intensity was magnified to display OAD fragment ions.

Using OAciD fragmentation, CID fragment ions provided evidence for the fatty acid composition while OAD specific fragment ions showed the potential to separate coeluting isomers at the MS/MS level. Using this approach, 30 unsaturated TGs were structurally characterized in the milk extracts (examples of higher mass TGs are shown in Fig. 3). In higher organisms, fatty acid double bond positions are typically found in the n-3, n-6 or n-9 positions however in ruminant species the n-5 and n-7 vaccenic acid are also commonly observed due to the influence from the gut microbiome.

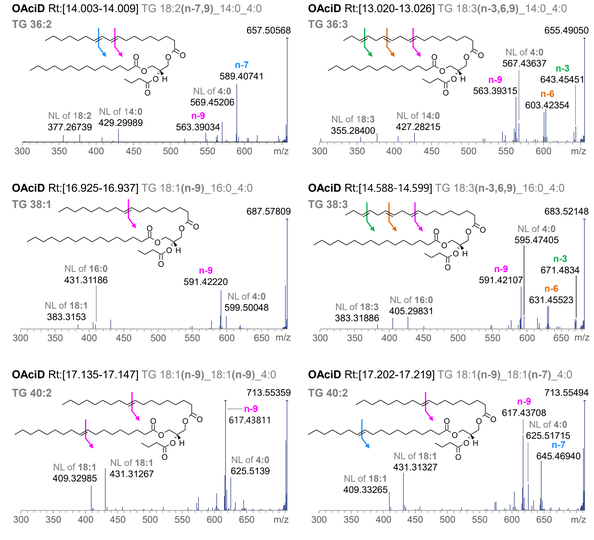

Figure 3. Examples of higher mass TGs annotated using OAciD combined fragmentation. Spectral intensity was magnified to display OAD fragment ions.

4. Conclusions

- OAciD-MS/MS was used to generate fragmentation data sufficient to annotate TGs at the fatty acid composition level as well as determining the C=C double bond position(s).
- OAciD enabled a highly specific fragmentation resulting in selective detection and high reporting confidence.
- TGs in a complex food commodity could be annotated with far greater specificity using OAciD-MS/MS compared to CID-MS/MS.