Analysis of triacylglycerol regioisomers in plant oils using direct inlet negative ion chemical ionization tandem mass spectrometry

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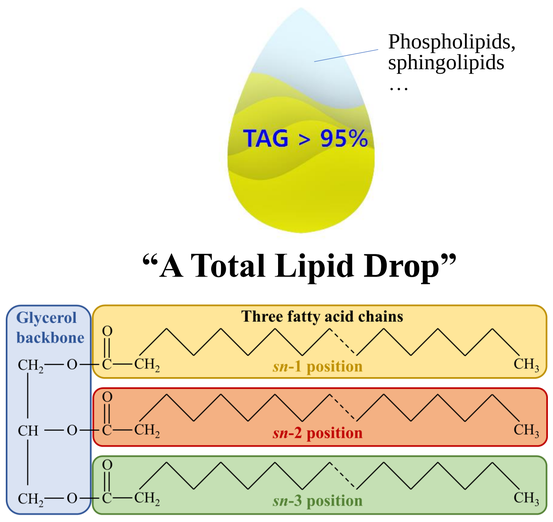
WHY DO WE NEED INFORMATION ON TAG REGIOISOMERS?



Triacylglycerol (TAG) molecular structure determines physicochemical and metabolic characteristic of oils.

AIMS

III. To provide guidance for further relevant research on fat- or oil-based products (e.g. infant formulas)

II. To verify the applicability of previous developed mass spectrometry method in complex TAG pools.

I. To comprehensively analyze the abundance and regiomeric compositions of TAGs in 18 plant oils.

STEPS

18 plant oils

• Extraction of total lipids (MeOH, $CHCl_3$, KCl)
• Isolation of TAG fraction (solid phase extraction)

GC-FID analysis of fatty acids (FAs) as FA methyl esters

MS analysis of TAG molecular weight distribution

MS/MS analysis of TAG regioisomers

Data handling and calculations

INSTRUMENT

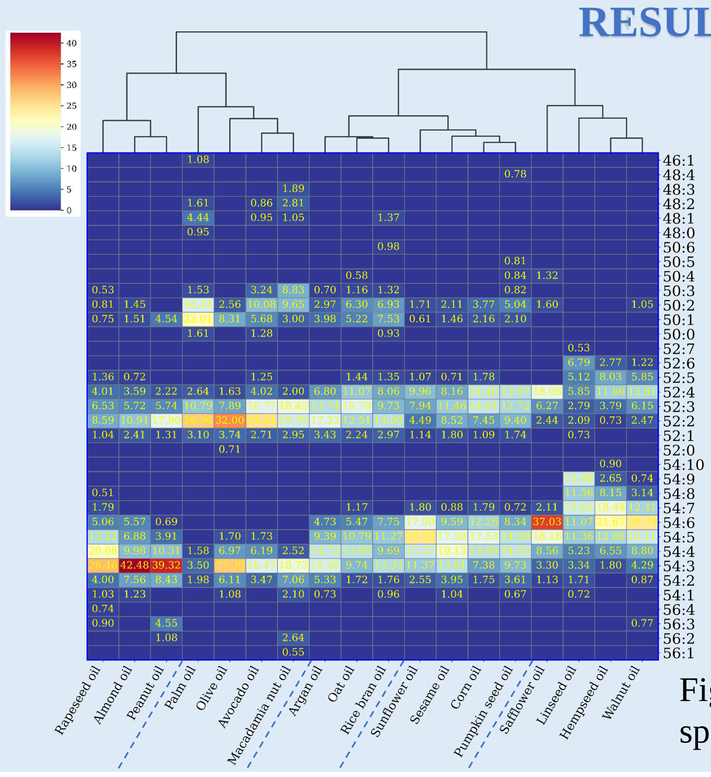
Thermo Scientific TSQ 8000 EVO mass spectrometer

Rapid direct inlet negative ion chemical ionization tandem mass spectrometry

(NICI-MS/MS)

- Without chromatographic separation
- Can determine the fatty acids in *sn*-2 and *sn*-1/3 positions
- Short analysis time (within 1 min)

RESULTS



Molecular weight distribution

Totally 35 molecular weight (ACN:DB, acyl carbon number : double bond) species were detected (Fig. 1). The molecular weight distribution in selected plant oils mainly presented with in the range of even-number ACN 46-56, and concentrated on ACN 50, 52 and 54.

Oils were grouped into 5 groups with clustering analysis for convenient discussion, which based on the similarity of TAGs molecular weight distribution.

Figure 1. Clustering analysis of 35 ACN:DB species of plant oils.

Regioisomer results

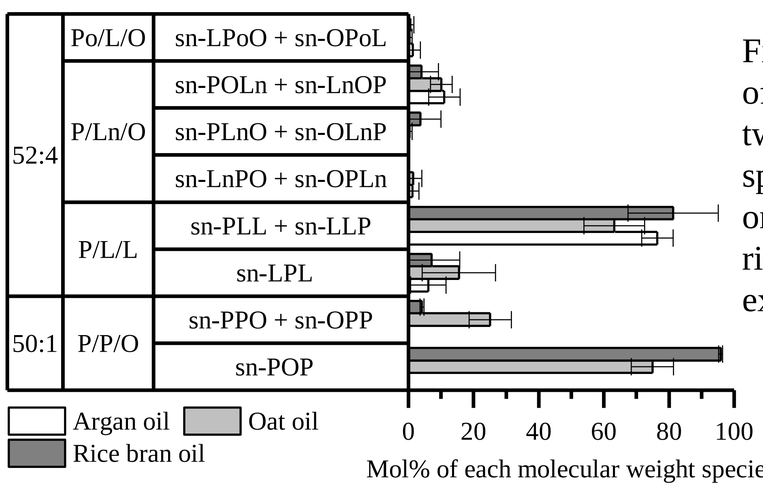


Figure 2. Molar percentage of TAG regioisomers in two example ACN:DB species (52:4 and 50:1) of one group (argan, oat and rice bran oils) as an example.

Abbreviations in Figure 2
L, linoleic acid,18:2
Ln, α/γ-linolenic acid, 18:3
O, oleic acid, 18:1
P, palmitic acid, 16:0
Po, palmitoleic acid, 16:1

Figure 2 shows one group (argan, oat and rice bran oils) as an example. For example in 52:4, the major regio-isomeric pair was PLL/LPL, which PLL (P in *sn*-1/3) was the most abundant TAG (63.2-88.5 mol%). Within both 52:4 and 50:1 TAG species, oat oil had more TAG regioisomers with P in *sn*-2 position compared with the other two oils. From all oil samples TAGs with molar percentage > 1% (totally 33 molecular weight species) were selected to MS/MS analysis to study regioisomerism. Totally, 220 TAG regioisomers were detected.

FA distribution profiles

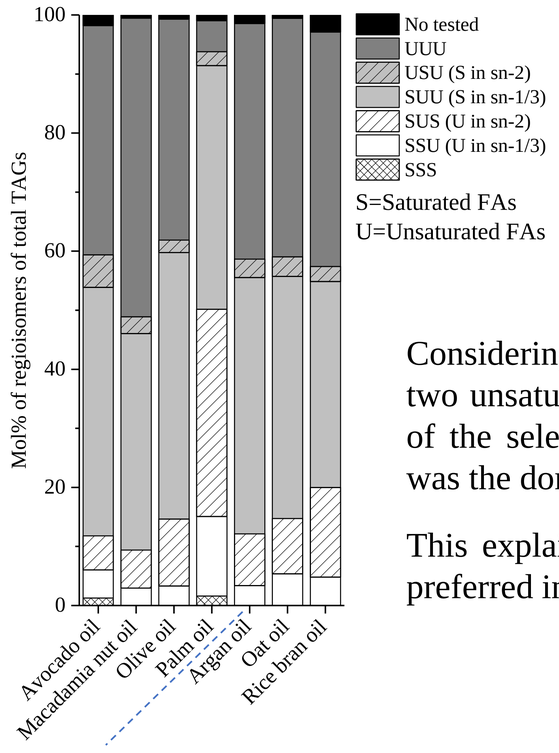


Figure 3. Molar percentage of regioisomers of total TAGs expressed as saturated and unsaturated FAs (two groups contained seven various oils as an example).

Considering both SFAs and UFAs, most of the TAGs with two unsaturated FAs accounted for major amounts in most of the selected oils, in which SUU (S in *sn*-1/3 position) was the dominating FAs (Fig. 3).

This explained why L and O were always in *sn*-2, and P preferred in *sn*-1/3 positions.

FAs distribution and abundance in TAGs

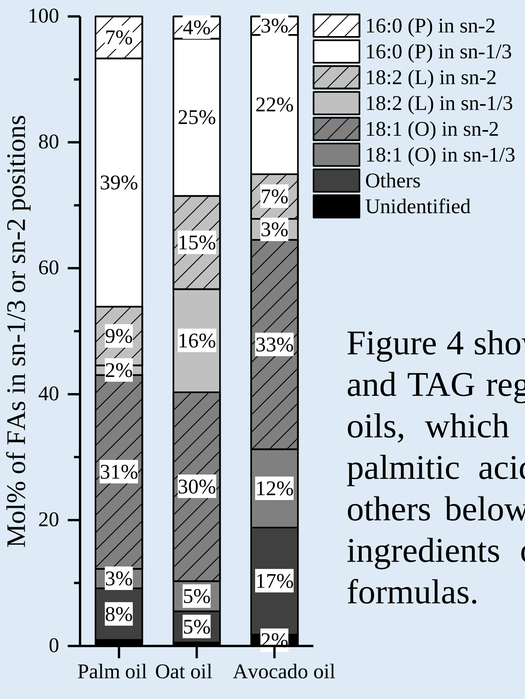


Figure 4. Molar percentage of three main FAs in *sn*-1/3 or *sn*-2 positions in three oils, which contained more palmitic acid in s*n*-2 compared to other oils.

Figure 4 shows combined molecular weight distribution and TAG regioisomer results of palm, oat, and avocado oils, which had considerable amount of TAGs with palmitic acid in *sn*-2 position (3-7% compared with others below 3%), indicating potential of these oils as ingredients of human milk fat substitutes for infant formulas.

CONCLUSION

- Totally 18 FAs, 35 molecular weight species and 220 TAG regioisomers were detected. Oleic acid (18:1, O), linoleic acid (18:2, L), and palmitic acid (16:0, P) were the three most abundant FAs resulting in TAGs of ACN 50, 52, and 54 as the most abundant TAGs in all the 18 kinds of selected plant oils.
- Unsaturated FAs were more commonly distributed in *sn*-2 position compared with saturated FAs, which is common for plant oils. However, palm, avocado, and oat oils differed from other oils in notable palmitic acid content in *sn*-2 position.

This high-throughput NICI-MS/MS method without need of chromatographic separation is suitable for determining regioisomers in complex TAG pools.

The results should be a constructive reference of further relevant research on fat- or oil-based products, for which plant oils are considered to be ideal and universal ingredients, such as infant formulas, dairy alternatives and food supplements.

ACKNOWLEDGMENTS

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